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NOEL FINLEY THOMPSON
1891–1944

JAMES G. DICKSON

Noel Finley Thompson, Associate Plant Pathologist, Wisconsin Department of Agriculture, died April 27, 1944. He had known for years that leukemia would terminate his work yet in his characteristic tranquil and confident manner he gave full service to the State and prepared for the final day. Natural teacher, inquisitive investigator, and honest administrator, he elevated regulatory work to cooperation in insect and disease control. His passing is a pronounced loss to phytopathology, the State of Wisconsin, and his wide circle of associates and friends.

Mr. Thompson was born at Oakland, California. The family later moved to Yakima, Washington, where he completed high school in 1910. He attended Whitworth College three years, transferred to the University of Washington, and completed the requirements for the B.S. degree in 1915. He held a teaching fellowship in botany at the University and the Puget Sound Biological Station in 1916 and 1917 and completed the requirements for the M. S. degree in Botany in 1916. He served six months in the armed forces in 1918. Upon release he was appointed Assistant Pathologist in the Office of Cereal Investigations, United States Department of Agriculture, on wheat smut eradication in the Pacific Coast area. In 1919 he accepted a summer appointment as instructor and Acting Head of the Botany Department at his Alma Mater and served as Assistant Professor of Botany at the University of Idaho the following year. He entered the graduate school, University of Wisconsin, in the autumn of 1920 and simultaneously assumed the responsibility for the barberry eradication program in the State. His characteristic devotion to his job soon resulted in a full-time appointment in barberry eradication, first in the Bureau of Plant Industry and later in the Bureau of Economic Entomology and Plant Quarantine, United States Department of Agriculture. In 1927 he joined the staff of the Wisconsin State Department of Agriculture and in this capacity served well the nurserymen, florists, cranberry growers, and other plant culturists until the time of his death.

On December 23, 1922, he married Grace Vivian Bitterman of Madison, Wisconsin. His wife and three children, Noel James, in the armed forces, Ellen Grace, and Elizabeth Anne, survive. Mr. Thompson was a member of the American Phytopathological Society, the Wisconsin State Horticultural Society, the Wisconsin State Gladiolus Society, the Wisconsin Academy of Science, Sigma Xi, Gamma Alpha, and Phi Sigma.

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USTILAGO STRIAEFORMIS. III. A FURTHER STUDY OF FACTORS THAT INFLUENCE AFTER-RIPENING OF CHLAMYDOSPORES FROM POA PRATENSIS[1]

K. W. KREITLOW[2]

(Accepted for publication September 18, 1944)

INTRODUCTION

In an earlier paper (15) on chlamydospores of *Ustilago striaeformis* (West.) Niessl in smutted leaves of *Poa pratensis* L., it was shown that treatment in a moist chamber at 32° to 35° C. reduced the after-ripening period of the spores from 197 days to less than 30.

Although races of *Ustilago striaeformis* with germinable chlamydospores have been found (2, 6, 14), in the vast majority of collections tested, only a negligible percentage of the spores germinated. Davis (3) succeeded in germinating spores of the smut only after they were exposed to a prolonged after-ripening period. Similar after-ripening periods of varying duration have been described for chlamydospores of other smuts. Since Noble (19) reviewed much of the literature on factors influencing germination of smut spores, only the more recent work will be discussed.

REVIEW OF LITERATURE

Many workers have found that freshly gathered spores of some smuts fail to germinate as readily as spores kept for varying periods at laboratory temperature (13, 17, 24). Holton (10), working with a race of *Tilletia tritici*, failed by several different treatments to induce germination of the typical, dark chlamydospores, while hyaline spores among the dark chlamydospores were capable of immediate germination. In contrast, Rump (23) found that dark chlamydospores of *Ustilago hordei* germinated readily while light brown or hyaline spores failed to germinate.

Several investigators have studied the stimulatory effect of light, gases, chemicals, presence of plant tissues, and temperature on germination of chlamydospores of different smuts. Light stimulated germination of spores of *Tilletia tritici* (9, 22), but if the spores were suspended in a medium that contained certain nitrogenous salts, light was not necessary. Ling (16) found that spores of *Urocystis occulta* germinated better in darkness or diffuse light.

The presence of oxygen was necessary for germination of spores of *Ustilago avenae* (12), *U. zeae* (21) and *U. hordei* (25). Concentrations of carbon dioxide up to 15 per cent also had a stimulatory effect (20). Davis (3) found that spores of *U. striaeformis* exposed to fumes of chloroform and

[1] Contribution No. 66, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the northeastern states.

[2] Associate Pathologist.

then submerged in a citric acid solution germinated earlier than untreated spores. A similar response was secured when spores of several smuts were exposed to ether vapors (5).

Chemical agents such as salts of light metals in weak concentrations and nitrogenous salts stimulated germination of chlamydospores of *Tilletia tritici* (9, 22). Spores of *Urocystis tritici* (19) and *U. occulta* (16, 26) germinated readily after treatment in a weak solution of benzaldehyde.

Crushed or uninjured plant tissues of different kinds as well as germinating seeds have a stimulatory effect on germination of chlamydospores of *Urocystis tritici* (8, 18, 19), *Ustilago avenae* (4), and *U. zeae* (20).

Spores of several species of *Urocystis* were induced to germinate after soaking in water at low temperatures for varying periods (7, 11). Kreitlow (15) shortened by several months the after-ripening period of spores of *Ustilago striaeformis* from *Poa pratensis* by incubating detached smutted leaves at 35° C. in a moist chamber. Since effective study of *U. striaeformis* is dependent on securing an abundant supply of germinable chlamydospores, further efforts were made to determine the nature of the response of chlamydospores to the incubation treatment.

MATERIALS AND METHODS

Chlamydospores were collected from two smutted clones of *Poa pratensis* propagated from single tillers and maintained in a greenhouse. The composite sample used in some of the tests was composed of the pooled smutted leaves of five different clones of Kentucky bluegrass. The chlamydospores were obtained by scraping spores from mature sori with a flattened needle or by macerating smutted leaves in water in a Waring Blendor (1).

Germination of chlamydospores was tested in quadruplicate by suspending spores in drops of distilled water on microscope slides (14). Each slide was placed in a Petri-dish moist chamber and the chlamydospores were incubated 16–20 hours at 25° C.

Methods for testing the factors considered likely to hasten after-ripening of spores are discussed under each heading. The factors studied were: Contaminating organisms, host tissue, abrasion or scarification of chlamydospores, temperature, and moisture.

EXPERIMENTAL RESULTS

Influence of Contaminating Organisms on the After-ripening of Smut Chlamydospores.—Fresh, smutted leaves of *Poa pratensis* were cut into lengths of approximately one inch. The leaves were rinsed in a 1:500 solution of mercury bichloride in 50 per cent ethyl alcohol for 15 seconds and then thoroughly washed in several changes of sterile water. Following this treatment, the leaves were placed in sterilized moist chambers and incubated at 35° C. At periodic intervals, pieces of leaves were removed aseptically from the moist chambers and the spores were scraped from smut sori for a germination test.

Germinable chlamydospores were obtained from the surface-sterilized leaves 10 to 25 days after incubation was started. In some of the tests, the spores required a longer period to attain maximum germinability. In some cases germination failed to exceed 30 per cent, but this may have been due to a residual effect of mercury bichloride. The results indicated that contaminating organisms did not shorten the after-ripening period of the smut chlamydospores.

Influence of Host Tissue on the After-ripening of Smut Chlamydospores.—Fresh, smutted leaves of *Poa pratensis* were cut into 1-inch lengths, placed in several hundred cc. of distilled water and macerated for one minute in a Waring Blendor. Following this treatment, residual plant material was removed by straining the spore suspension through a piece of cheesecloth. Chlamydospores suspended in the plant juice and water were concentrated by centrifuging the suspension at low-speed for several minutes in an angle centrifuge. Most of the supernatant liquid was decanted and the spores were resuspended in the remaining liquid and collected by pouring the suspension on a fine grade filter paper.[3] That portion of the paper retaining the greatest concentration of filtered chlamydospores was cut into small bits and placed in a Petri-dish moist chamber for incubation at 35° C. At the same time a sample of the spores was tested for germination.

Another suspension of chlamydospores in plant juices and water was subjected to low-speed centrifugation and the spores were resuspended in sterile water. The cycle of washing and centrifuging chlamydospores was repeated three times. After each washing, some of the spores were resuspended in 10 cc. of sterile water and collected on filter paper for incubation at 35° C. Samples of spores for immediate germination tests were collected at each stage of the operation.

None of the chlamydospores removed at different stages of treatment for an immediate test proved germinable. Tests at frequent intervals on spores incubated at 35° C. in moist chambers revealed that spores washed several times were more germinable than unwashed spores or those washed only once. A germination of 35 to 50 per cent was obtained after incubation for 12 days with the spores washed three times compared with 25 per cent for spores washed once and 10 per cent for unwashed spores. Presence of host tissue, however, was not necessary for hastening after-ripening.

Abrasion as a Factor Hastening After-ripening of Smut Chlamydospores.—During the course of experiments to free chlamydospores of host tissue, it was observed that the after-ripening period for spores treated in the Waring Blendor frequently was reduced by several days. It seemed possible that the treatment may have scarified the spores and thus hastened germination.

Fresh, 10-gram samples of smutted leaves of *Poa pratensis* were placed in 400 cc. of distilled water and treated in a Waring Blendor for 15 seconds, 30 seconds, 60 seconds, 3 minutes, and 5 minutes. A control was prepared

[3] Schleicher and Schüll Co., Inc., New York, N. Y. No. 589, 12½ cm.

by scraping spores from leaves of the same plants into 50 cc. of distilled water. After each interval of treatment, a 50-cc. sample of the spore suspension was removed for concentrating and washing in the angle centrifuge. Each spore sample was washed three times and the spores collected on filter paper for incubation at 35° C. Germination of the treated spores was tested every other day.

Chlamydospores from two different clones and from a composite sample of five different clones of Kentucky bluegrass all responded similarly. Spores from the control required 10 to 15 days to after-ripen at 35° C. Most of the spores agitated in the blender after-ripened 3 to 10 days following treatment and incubation at 35° C.

There was no definite increase in germinability of chlamydospores with length of treatment time. Spores agitated 15 seconds seemingly germinated as rapidly and as well as spores treated five minutes. Conversely, there was no apparent injury from the longer treatment periods.

Agitating spores in the blender not only hastened germination but also gave a definite increase in germination over untreated spores. Whereas rarely more than 75 per cent of untreated spores germinated, often 90 per cent of treated spores germinated.

Influence of Temperature on the After-ripening of Chlamydospores Agitated in a Blender.—Chlamydospores were removed from fresh, smutted leaves of each of two clones of *Poa pratensis* by macerating the leaves in a Waring Blendor for one minute. The spores from each clone were washed three times in an angle centrifuge and collected on filter paper. Pieces of filter paper bearing spores from the separate clones were placed in Petri-dish moist chambers for incubation at 5°, 25°, and 35° C. Spores from each set of dishes were removed for an immediate germination test. Germinability of the spores was determined thereafter at 5-day intervals.

Tests with chlamydospores incubated at each of the three temperatures showed that after-ripening was hastened only in those spores stored at 35° C. Of chlamydospores stored at 5° and 25° C., less than 10 per cent germinated during a 30-day storage period, while those stored at 35° C. attained a maximum germinability, for this experiment, of 75 per cent within 15 days.

Effect of Storage at Lower Temperatures on Subsequent After-ripening of Chlamydospores.—Chlamydospores from each of the two clones of *Poa pratensis* used in the previous experiment were maintained in moist chambers at 5° and 25° C. for 60 days. The Petri-dish moist chambers containing the spores were then transferred to an incubator at 35° C. for after-ripening of the chlamydospores.

When spores were stored at 5° C. an after-ripening period of 10 to 20 days at 35° C. was necessary to secure maximum germination, while chlamydospores maintained at 25° C. before they were transferred to 35° C. required only 3 to 5 days to reach maximum germinability. This suggested that partial after-ripening may have occurred in the spores stored at 25° C.

Moisture as a Factor Influencing After-ripening of Smut Chlamydospores.—Chlamydospores were obtained from fresh, green, smutted leaves

of a single clone of *Poa pratensis* (Clone 37KB120(26)) by macerating the leaves in a Waring Blendor for varying lengths of time. The spores were washed three times, collected on filter paper, and placed, with a control, at different moisture levels in an incubator at 35° C. Spores stored dry were placed inside Petri dishes containing dry sheets of filter paper. Spores incubated partially dry were placed inside small, dry, open dishes which in turn were placed inside a Petri-dish moist chamber. This permitted the filter paper bits containing the spores to remain dry while the spores themselves were maintained in a humid atmosphere. Chlamydospores incubated wet were placed inside small covered dishes containing wet filter paper and these

TABLE 1.—*Germination of chlamydospores agitated for varying lengths of time in a Waring Blendor and incubated for after-ripening at 35° C. under different moisture conditions*

Agitation time	Moisture	Percentage germination 0 to 31 days after incubation							
		0	2	5	7	9	12	19	31
Check	Dry	.....	few	few–5	few–10	0–few	few	few	few
	Partly dry	.....	0–few	few	0–few	0	0	5	10–25
	Wet	few	few–5	15	50	90	90	50–75	10–25
15 sec.	Dry	.....	5	few–5	few–5	few	few–5	few	few
	Partly dry	.....	few	few	few	0	0–few	0–few	10–25
	Wet	few–5	few–5	50–75	90	75–90	90	90	50–90
30 sec.	Dry	.....	few–5	5–10	few–5	few	5	few	few
	Partly dry	.....	5–10	few–5	0–few	0–few	0	few	25–50
	Wet	5–10	10	75	30–50	90	90	90	90
60 sec.	Dry	.....	few–5	5	few–5	5	few	5	few
	Partly dry	.....	few	few	0–few	0–few	0	0–few	25
	Wet	few–5	few	25–50	75–90	75–90	75–90	75	10–25
3 min.	Dry	.....	5	few–5	5	few	0–few	few	few
	Partly dry	.....	few–10	few	0–few	0–few	0–few	0–few	10–25
	Wet	few–5	5	75–90	75–90	75–90	90	50	25–50
5 min.	Dry	.....	few	few	0–few	0–few	0–few	0–few	0
	Partly dry	.....	few–10	5–10	few–5	0–few	10–50	25–75	10–75
	Wet	few	15–30	75	90	90	90	90	75

dishes in turn were placed inside Petri-dish moist chambers. In this manner, the spores on the filter paper bits were in direct contact with moisture and were insured against drying-out at the high temperature. The dishes in each condition were examined daily and the water replenished when necessary. Germination was tested for spores in each environment at frequent intervals.

As shown in table 1, after-ripening of smut chlamydospores occurred when the spores were maintained wet at 35° C. Some after-ripening apparently occurred among spores stored under partially dry conditions since germinability had increased to 25 to 50 per cent after 31 days. Also, spores agitated five minutes began to after-ripen under partially moist conditions 10 to 15 days earlier than spores treated for shorter periods or not treated at all. Chlamydospores stored dry at 35° C. failed to after-ripen regardless of treatment.

DISCUSSION

Treatments have been found that reduce the after-ripening period of the chlamydospores of stripe smut on *Poa pratensis* from 197 days to less than 30 days. The prolonged soaking used by Davis was replaced by incubating chlamydospores at 35° C. in a moist chamber. Since many factors probably are involved in after-ripening, an effort was made to determine which were primarily responsible.

The influence of contaminating organisms was eliminated when spores within surface-sterilized host tissues were made germinable in a reasonable time by proper incubation treatment. Presence of host tissue was found unnecessary since spores washed free of plant juices and after-ripened on filter paper were germinable after a short incubation period. While freeing spores from host tissue by macerating smutted leaves in a Waring Blendor, it was observed that spores so treated frequently germinated more abundantly and earlier than untreated spores, possibly because of abrasion or scarification of the spores. Chlamydospores treated in the blender, however, still required incubation at 35° C. in a moist chamber before they became germinable.

Partial after-ripening may occur when spores are stored at certain temperatures. Spores stored moist at 25° C. for three months after-ripened in 3 to 5 days when incubated at 35° C. In contrast, spores stored at 5° C. required 10 to 20 days' incubation.

The importance of adequate moisture was demonstrated when spores incubated dry at 35° C. failed to after-ripen while spores incubated in contact with free water reached maximum germinability in a short time. Chlamydospores incubated in a saturated atmosphere but not in direct contact with water after-ripened only partially. It was observed that few spores germinated during the incubation treatment at 35° C. despite intimate contact of spores and water. Germination occurred only after treated spores were placed in drops of water and incubated at a lower temperature for 16 to 20 hours.

SUMMARY

Chlamydospores of *Ustilago striaeformis* from *Poa pratensis* were after-ripened in host tissue free of contaminating organisms. Spores washed free of host tissue were after-ripened on filter paper bits incubated at 35° C. in a moist chamber.

Treatment of chlamydospores in a Waring Blendor hastened after-ripening and enhanced germinability.

Fresh chlamydospores stored at 5° C. for 60 days and then transferred to 35° C. required longer to after-ripen than a similar set of spores stored at 25° C.

Water in contact with the spores was necessary for successful after-ripening. Spores incubated in a saturated atmosphere required longer incubation, while spores incubated dry failed to germinate.

UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY,
STATE COLLEGE, PENNSYLVANIA.

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SYNTHETIC CULTURE MEDIA FOR THE ROOT-ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM[1]

WALTER N. EZEKIEL[2]

(Accepted for publication October 15, 1944)

In studies (6) on nutritive relations of *Phymatotrichum omnivorum* (Shear) Duggar, a synthetic culture solution designated as "solution 70" was derived by successive modification of Brown's artificial potato-dextrose medium (2). Later, solution 70 proved a convenient basic medium for testing additions of either nutrient or possibly inhibitory materials (1, 5, 13). Talley and Blank (13) found that this solution was well balanced and that growth of the fungus was not improved significantly by increasing or decreasing any of the major inorganic components. Growth increased with additional nitrogen (as ammonium nitrate) and carbon (as dextrose) but efficiency of carbon utilization decreased.

Several workers (1, 7, 10, 11) proved that substrata for *Phymatotrichum omnivorum* were improved by additions of zinc, iron, manganese, and sometimes copper. In extensive experiments, Blank (1) found that with unpurified solutions, greater growth was obtained with additions of salts of zinc, iron, and manganese to provide about 2 ppm. of these metals. With solutions purified with calcium carbonate, copper was beneficial also, but additions of copper to unpurified solutions were sometimes detrimental.

The results summarized in table 1 illustrate the relative importance of these metals in the nutrition of *Phymatotrichum omnivorum*. Traces of heavy metals were first removed from solution 70 by the Steinberg (12) technique (autoclaving with calcium carbonate and filtering) and salts of the metals then added. Growth was initiated from agar discs cut from plate cultures of *P. omnivorum*, isolate 57, grown on 135-agar (6). In agreement

TABLE 1.—*Growth of Phymatotrichum omnivorum in purified solution 70 with the designated additions of heavy metals. (Mean values of quintuplicate cultures in 25 ml. portions of substrata, incubated at 28.5° C.)*

Composition of nutrient solutions, indicated as additions to purified solution 70	Mean dry weight of colonies, after		
	3 weeks	4 weeks	5 weeks
	mg.	*mg.*	*mg.*
No additions (lacks Zn, Fe, Mn, Cu)	35	35	54
Plus 2.5 ppm. Fe, Mn, Cu (lacks Zn)	41	54	67
do Zn, Mn, Cu (lacks Fe)	119	141	192
do Zn, Fe, Cu (lacks Mn)	232	287	307
do Zn, Fe, Mn (lacks Cu)	251	314	333
do Zn, Fe, Mn, Cu; complete[a]	292	336	338

[a] This complete solution is designated subsequently as solution B.

[1] Published with the approval of the Director as Technical Contribution No. 860 of the Texas Agricultural Experiment Station.

[2] Formerly Pathologist, Texas Agricultural Experiment Station; now Senior Mycologist, Bureau of Ordnance, Navy Department.

with the results of Blank (1), lack of zinc reduced growth most sharply, lack of iron somewhat less, and lack of manganese or copper to a still lesser extent. (By contrast, additions of gallium to the complete solution had little effect on growth of the fungus.)

In recent work, the various synthetic media have generally been used with additions of the metals. For convenient reference, and to avoid the continuing circumlocution of repeatedly describing these media as deviations from the old formulas, the more important formulas as now used are assembled below.

In preparing these media, additions of the small amounts of heavy metals are conveniently made from stock solutions prepared so that 1-ml. portions will furnish 2.5 ppm. for 1 liter of final culture solution. For 100-ml. volumes of the stock solutions, use respectively: $ZnSO_4 \cdot 7H_2O$, 1.10 g.; $Fe_2(SO_4)_3$, 0.90 g.; $MnSO_4 \cdot H_2O$, 0.77 g.; and $CuSO_4 \cdot 5H_2O$, 0.98 g.

Solution A is essentially the old solution 70 with Zn, Fe, and Mn added. The basic solution contains per liter:

$MgSO_4 \cdot 7H_2O$	0.75 g.
K_2HPO_4	1.35 "
KCl	0.15 "
Ammonium nitrate	1.18 "
Dextrose	40.0 "

Zn, Fe, and Mn sulphates are added in amounts to furnish 2.5 ppm. of each metal.

Solution B is the same as solution A, except that the basic solution is purified (by autoclaving with 15 g. of calcium carbonate per liter for 20 minutes at 15 lb. and filtering while hot) and addition then made of Cu as well as Zn, Fe, and Mn, each at 2.5 ppm. This solution has been used, for example, in testing possible growth-promoting materials (4) and in measuring the inhibitive effect of various compounds (8, 9).

Solution C, modified from the old solution 81 (6), provides a more highly buffered solution than A or B, and has been used in study of pH ranges of isolates of the fungus. It contains per liter:

$MgSO_4 \cdot 7H_2O$	1.0 g.
K_2HPO_4	2.0 "
Asparagin	2.0 "
Peptone	2.0 "
Dextrose	40.0 "

plus 2.5 ppm. each of Zn, Fe, and Mn.

Agar D is based on 135-agar (6), which was useful as a general culture medium for *Phymatotrichum omnivorum* and some other fungi. For stock cultures and in various experiments (3), agar D has proved better with *P. omnivorum* than potato-dextrose agar. This medium contains per liter:

$MgSO_4 \cdot 7H_2O$	0.75 g.
K_2HPO_4	1.5 "
Peptone	4.2 "
Ammonium nitrate	1.2 "
Sucrose	40.0 "

plus 2.5 ppm. each of Zn, Fe, and Mn, plus agar 20 g.

Agar E. Copious development of sclerotial masses around walls of flask cultures has been obtained with 134-agar (6), which differed from 135-agar by inclusion of 40 g. per liter of commercial corn starch. With the further addition of the heavy metals, this is now designated as agar E. Make up as agar D, but incorporate 40 g. of starch before adding the agar. (The starch is conveniently first stirred into about 100 ml. of cold water, mixed next with about 400 ml. of boiling water, then with the other ingredients which have been dissolved in the remainder of the water, and the agar finally added.)

AGRICULTURAL EXPERIMENT STATION,
COLLEGE STATION, TEXAS.

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ROOT DISEASE OF CASTANEA SPECIES AND SOME CONIFEROUS AND BROADLEAF NURSERY STOCKS, CAUSED BY PHYTOPHTHORA CINNAMOMI

BOWEN S. CRANDALL,[1] G. F. GRAVATT,[2] AND MARGARET MILBURN RYAN[2]

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INTRODUCTION

Soon after extensive work on chestnut blight was started in 1912, reports of a disease of chestnut were received from regions where it seemed unlikely that blight had yet gained a foothold. Dying chestnuts, *Castanea dentata* (Marsh.) Borkh., were reported at the lower elevations in the southern Appalachians from Virginia to Georgia, and in the mountainous and hilly regions of west Tennessee, north Alabama, and north Mississippi. Observations by various individuals disclosed that the dying was caused by a root disease. It was first supposed that *Armillaria mellea* Fr. was responsible, since it was the only pathogenic organism isolated from the rotted tissue. In 1931, using new techniques, a *Phytophthora* was isolated by the writers from the roots of dying American chestnut and chinkapin trees and was eventually identified as *P. cinnamomi* Rands and so reported by Tucker (33). The same fungus was causing a destructive root rot of forest-tree nursery stock. This paper reports the work leading to the identification of the root-rotting *Phytophthora* as *P. cinnamomi* and the inoculation and field tests that proved its pathogenicity on chestnut and other hosts.

HISTORY OF THE PARASITE

For over 100 years chestnut trees (*Castanea sativa* Mill.) in Europe have been dying from a root rot called the "ink disease." It was first reported from Portugal in 1838 and elsewhere in Europe soon after, but no detailed studies prior to those of Gibelli (15), about 1879, are known. Numerous other pathologists, especially French and Italian, tried to discover the cause of the disease, which they attributed, among other things, to *Armillaria mellea, Coryneum perniciosum* Briosi and Farneti, mycorrhizal fungi that had become parasitic, bacterial complications, and poor soil conditions (12). Finally, about 1917, Petri isolated the true causal organism and classified it as *Blepharospora cambivora* Petri (26), later changed to *Phytophthora cambivora* (Petri) Buis. Later *P. cinnamomi* Rands also was reported associated with the "ink disease" of chestnut and beech (*Fagus sylvatica* L.) in England (11) and chestnut trees in southern Europe (22).

[1] Associate Pathologist, Office of Foreign Agricultural Relations; formerly Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Athens, Ga., in cooperation with the School of Forestry, University of Georgia, Athens, Ga.

[2] Respectively, Senior Pathologist, and formerly Assistant Scientific Aide, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Plant Industry Station, Beltsville, Maryland.

In the United States the record of dying *Castanea* can be carried back to 1824 with some certainty. In that year chinkapins were reported dying from unknown causes in Georgia (18). The general recession of the chestnut trees in the Southern States has been reviewed by Clinton (4). Reports of old residents described the dying of chestnut and chinkapin trees at lower elevations in north Mississippi, Alabama, Georgia, Tennessee, Virginia, Maryland, and the Carolinas. Around 1900 the Ozark chinkapin (*Castanea ozarkensis* Ashe) was dying in Arkansas, Missouri, and Oklahoma except on the tops of high, dry ridges in the Ozarks.

In 1930 *Phytophthora cinnamomi* was first reported in the United States by White (37) on rhododendron. No *Phytophthora* was reported on *Castanea* in this country until 1932 when Milburn and Gravatt (24) isolated a *Phytophthora* from dying *C. dentata* and *C. pumila* (L.) Mill. This *Phytophthora* later was identified as *P. cinnamomi* Rands. In 1933 *P. cinnamomi* was found to be the cause of a destructive root rot of red pine (*Pinus resinosa* Ait.) in nursery seedbeds (16). A number of coniferous and broadleaf seedlings and transplants later were found to be subject to root rot in the nursery or in plantings (6). Before the discovery that *P. cinnamomi* was responsible, the losses were attributed by some nurserymen to high water table and by others to heavy soils. These conditions favor attack by *Phytophthora.* According to the files of the Division of Mycology and Disease Survey, Beltsville, Md., *P. cinnamomi* was reported by Plakidas in 1939 on tung in Louisiana and by Wager in 1940 on avocado in southern California. In 1938 *P. cambivora* was first reported in the United States, on maple (*Acer platanoides* L.) in New Jersey (29). The authors never have found it associated with root rot of chestnut or nursery stock in the United States.

In 1936 Mehrlich (23) questioned the validity of separating *Phytophthora cinnamomi* Rands from *P. cambivora* and suggested that "they be combined into a single species *P. cambivora* in which strain differences may be recognized." In 1937 White (38) placed the rhododendron wilt fungus in the combined species *P. cambivora.* In 1936 the name *P. cambivora* (*P. cinnamomi*) was used in reporting root rot of nursery stock caused by *P. cinnamomi* (6). Some confusion has resulted from this combination of species. The two species, as separated and recognized by European authorities and by Tucker in this country, are present in the United States. The writers are keeping the two species separate.

SYMPTOMS

Above-ground symptoms of this root disease vary considerably. In some cases in early summer, typical wilting of the leaves appears, followed by death and defoliation of the tree. In other, more typical cases the slow loss of roots is first indicated by a gradual reduction in the size of the leaves over a period of several years (Fig. 1, B). This condition usually is accompanied by chlorosis and slight wilting. In some such cases the entire tree dies during the dormant season. In other cases the branches die back until

only a few are left; these may remain alive for several years (Fig. 1, C and D).

The symptoms of the disease on and in the roots are similar to those

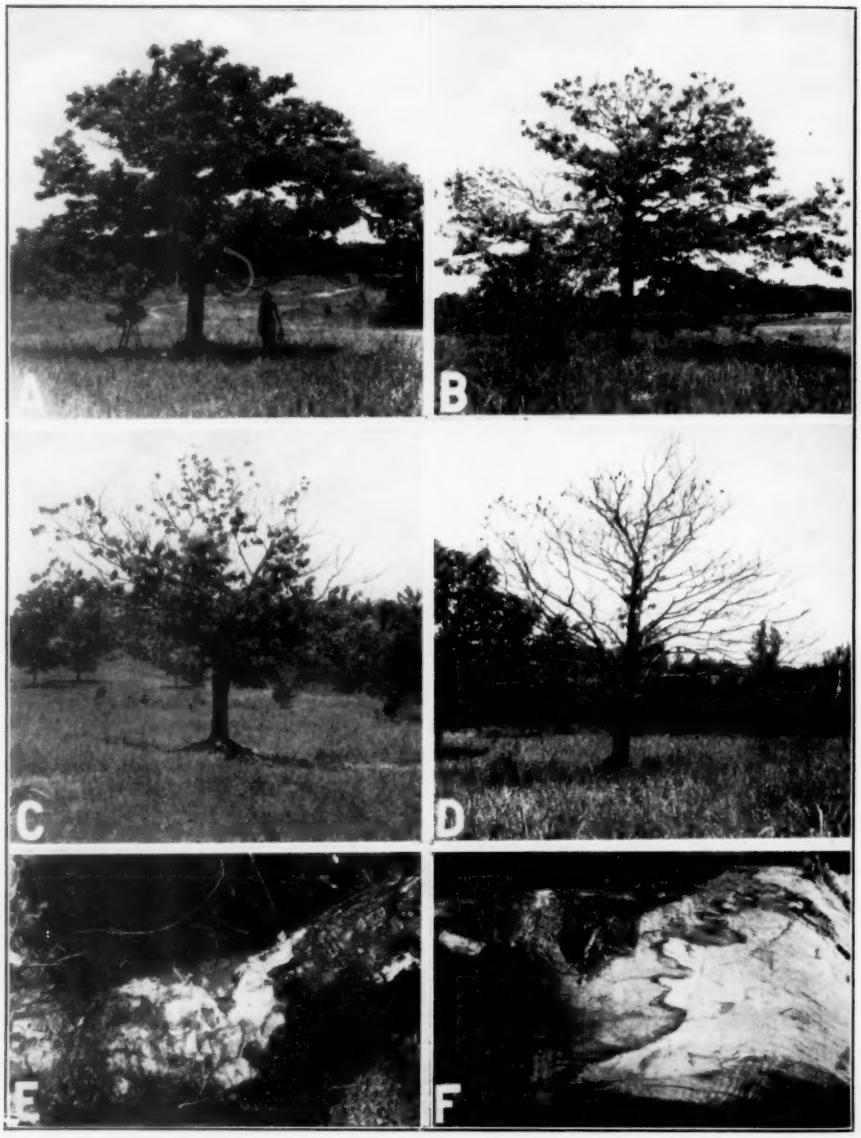

FIG. 1. A. Healthy-appearing chestnut tree. B. Early symptoms of root rot. C and D. Advanced symptoms of root rot. E. Surface symptoms of root-rot lesion. F. Dissection, showing advancing wedge-shaped streaks.

described in Europe for the "ink disease." The lesions on the roots usually may be detected by the presence of an inky-blue exudate that stains the soil close to the root. The surface of a lesion is brownish-black (Fig. 1, E).

The lesions vary from small round spots to areas extending a number of feet along a root. Eventually, the lesions coalesce and girdle the root, and the tree dies when the collar is girdled or when most of the roots are killed. Often the first lesions are on the taproot well below the root collar.

When a lesion is dissected, recently invaded tissue is commonly mottled light brown and green with no definite margin. The fungus generally advances from the invaded region into sound tissue in irregular, wedge-shaped streaks (Fig. 1, F). While essentially a root parasite, the causal *Phytophthora* can often be found above the ground line in the collar region.

EPIDEMIOLOGY

Recession of the American Chestnut

Early in the study of the chestnut root rot it became evident that the facts being brought to light probably accounted for the steady recession of the chestnut from large areas in the Gulf and Atlantic States to the foothills and mountains of Mississippi, Alabama, Georgia, Tennessee, Maryland, Virginia, and the Carolinas. Buttrick (3), in a report based on field work done in 1912–1913, states, "During the last seventy-five years the range of chestnut in North Carolina has decreased considerably. It was formerly found throughout the western Piedmont section, and remains of old stands and single isolated trees are still to be met with. Along the eastern slopes of the Blue Ridge and occasionally at the lower elevations in the mountains themselves, the recession may still be observed going on, trees are in poor health and dying off and their places are being taken by other species. The cause of this recession is not well understood, though various reasons have been advanced."

We are accustomed to thinking of introduced epidemic diseases as something more or less peculiar to this century, characterized as it is by speedy means of communication. The history of *Phytophthora cinnamomi* as we have been able to reconstruct it indicates that the fungus probably came to the United States more than a hundred years ago, possibly by way of trading ships operating between one of our southern ports and the East Indies or Asia. The importation of numerous exotic plants for the gardens of antebellum estates could easily have been the direct means of its entry. Once introduced, it apparently spread slowly westward, northward, and inland, its hosts being the American chestnut and chinkapins. Some observers noted the dying of northern red oaks (*Quercus borealis* Michx. f.) where this species occurred mixed with dying chestnut and chinkapins. This species, although highly susceptible in nurseries, has not been proved a host for *P. cinnamomi* in forest stands.

In the course of the survey work on the disease, numerous reports were obtained from old residents giving the story of the death of the chestnuts and chinkapins, either as they remembered it or as it was told to them.[3] An

[3] A resident from McCutchins Cove in the Cumberland Mountains of Tennessee stated that he remembered seeing the floor of the valley covered with a heavy stand of chestnut. All chestnuts suddenly sickened and died within a year or two, except a few along the

interesting characteristic of the root disease, noted by many of the early observers and by the writers, is the simultaneous decline and death of large numbers of trees in one locality. Often almost all of the trees of these species in areas of many acres or in an entire valley died within 2 or 3 years. Isolated trees within these areas remained healthy-appearing for years. The dates of the disappearance of the chestnut and chinkapins as given in these reports and in literature are plotted on the map in figure 2. Shaded areas show the regions where the writers noted symptoms of the root disease. The ranges of the American chestnut and chinkapins are shown, and the locations where *Phytophthora cinnamomi* has been isolated are marked.

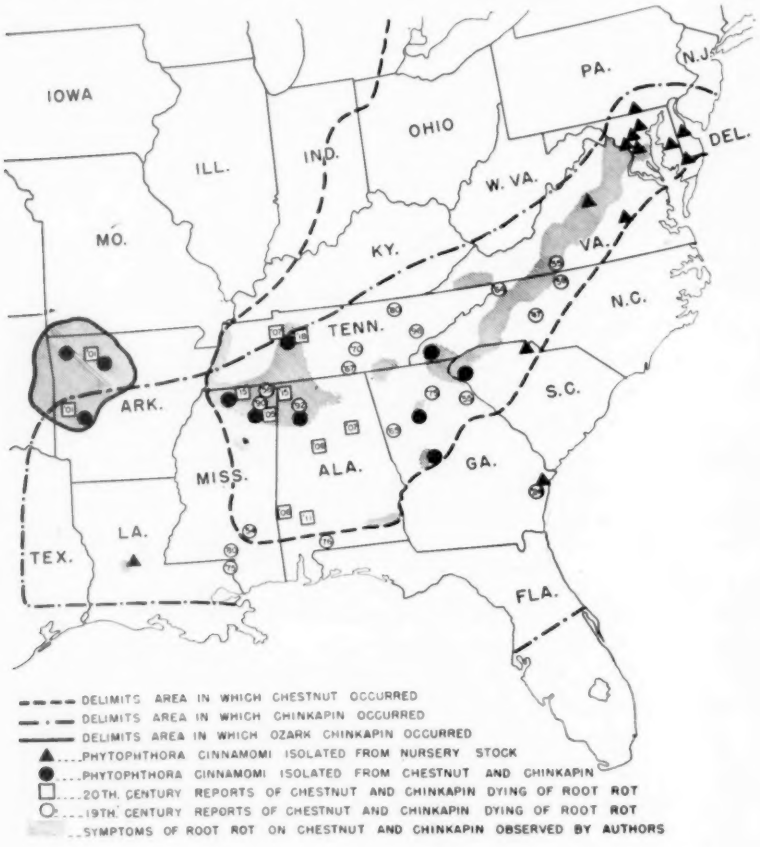

FIG. 2. Observed range of root rot caused by *Phytophthora cinnamomi*.

Recession of the Ozark Chinkapin

About 1900 a duplication on a limited scale of what probably happened to the chestnut occurred through the range of the Ozark chinkapin. Reports

tops of the hills. He placed the date accurately as 1867, since it was the year when soldiers were returning from military prisons in the North, and the residents of the Cove thought that the disease was something carried from the Yankee prisons by the returning soldiers.

of residents and Forest Service personnel then stationed in the area indicate that most of the chinkapin population on bottom land and at low elevations died within a few years. The root rot is active on survivors of the original epidemic and the species is slowly disappearing. Only a few large mature trees remain, at high elevations in northwest Arkansas.

Local Spread

How the *Phytophthora* originally reaches an area is not known, but various means of transfer are possible. As soon as *Phytophthora* appears in an area, the trees growing on the lowest, poorest-drained sites are the first to die. Those on heavy soils are affected first. The fungus then spreads from tree to tree from the lower areas to the higher dryer areas. Trees on loose, sandy, well-drained sites frequently remain unaffected for years. On moist sites with heavy soils there is some evidence that the fungus infects the roots at numerous places after spreading through the soil from the original infection court.

ISOLATION FROM CASTANEA SPECIES

Late in 1931 specimens of roots from dead and dying American chestnut trees were sent to the Division of Forest Pathology from an orchard in Georgia. Cultures from the affected areas yielded a fungus identified as a *Phytophthora* and similar to *P. cambivora,* cause of the "ink disease" in Europe. However, as certain differences were noted, comparisons of this chestnut root rot fungus with *P. cambivora* and *P. cinnamomi* were made. This same *Phytophthora,* subsequently determined to be *P. cinnamomi,* was isolated from American chestnut specimens from other localities in Georgia and Tennessee, from *Castanea pumila* in South Carolina, and from *C. crenata* Sieb. and Zucc. in Louisiana. In 1935, in a survey made through the ranges of the various species of *Castanea* in the Southern States, *P. cinnamomi* was isolated from American chestnuts dying of root rot in Mississippi and Alabama, from Ozark chinkapin in Arkansas, and from the Alabama chinkapin (*C. alabamensis* Ashe) in Alabama.

The *Phytophthora* appeared in only 4 per cent of the cultures from naturally infected specimens shipped in by mail. In the field, where isolations were made as soon as the roots were dug, the percentage was only slightly higher, but quite variable. The most important factor in the successful isolation of *Phytophthora* is to locate healthy tissue that is just being actively invaded. Under such conditions the pathogen is growing ahead of other fungi that overwhelm it in culture. The best times of the year for isolating the pathogen from diseased roots appear to be spring and fall; however, isolations have been made in every month of the year. Day (11) reported that in England *P. cinnamomi* was isolated from *C. sativa* only during September and November. Difficulty in isolating *Phytophthora* from walnut (31) and chestnut (13) has been attributed to the presence of toxic exudates as well as saprophytic organisms. In the present study, especially where

the specimens were shipped in by mail, molds and particularly *Trichoderma* were fruiting on the surface of the bark. *Trichoderma* has been found fruiting on *Phytophthora* lesions on roots at the time they were dug. Since *Trichoderma* has been reported parasitic on various soil fungi, including a species of *Phytophthora* (36), possibly it may be parasitic on the *Phytophthora* on chestnut roots. In tests, 9 of 12 isolates of *Trichoderma* found as contaminants in cultures where the chestnut *Phytophthora* was expected, apparently were able to parasitize *P. cinnamomi* in pure culture. However, nursery field tests in which the soil was inoculated with *Trichoderma* in an attempt to control *Phytophthora* gave negative results.

The best method for isolating *Phytophthora cinnamomi* was to place sections of diseased root tissue containing only the advancing edge of the rot and about one-fourth inch of healthy tissue in test tubes containing sterile water. Sections from chestnut should be kept in water for 2 or 3 days, several changes of water being desirable. The sections then should be lightly surface-sterilized and plated on corn-meal or prune agar. Typical growth of *P. cinnamomi* can often be detected in a few days.

A method suggested by Dr. C. M. Tucker, inserting rot sections in apple and subsequently isolating *Phytophthora* from the apple tissue, has been especially good for infected native species of *Castanea*. Sections of rot tissue are selected and surface-sterilized, and the outer bark is removed. Small slivers of wood and inner bark containing infected and healthy tissue are cut and forced into an apple through a cut in the skin. The hole should be sealed with tape or vaseline. This method fails at times because of contamination by the vigorously growing apple parasite, *Diplodia natalensis* P. Evans.

THE PATHOGEN

Description

Better growth was obtained on oatmeal agar made by Tucker's formula (32) than on any other medium employed; on it the fungus produced an abundant aerial growth of tangled, white hyphae and chlamydospore-like bodies. Similar growth occurred on sterilized carrot slants. On corn-meal agar there was little aerial growth and many of the hyphal tips were swollen and variously shaped (Fig. 3, C). The same kind of round bodies appeared on lima-bean agar.

The scant growth that appeared on steamed corn meal was tangled, white, and aerial, like that on the oatmeal agar. Medium-length aerial hyphae with vesicles in the submerged growth occurred on carrot agar. In nutrient solution made according to Petri's formula (27), some vesicles were produced; however, they were more abundant in sterile tap water cultures (Fig. 3, B). The hyphae were hyaline and averaged 5.2 μ in width, with a range of from 4.7 to 6.2 μ. They had only occasional cross walls and contained many fat globules. The chlamydospore-like bodies (Fig. 3, A) were round and thick-walled, averaged about 29 μ in diameter, and usually appeared singly. The vesicles (Fig. 3, B) were irregular in shape and size,

were thin-walled, and occurred in bunches. Petri believed that vesicles act as conservation agents since the extraordinary abundance of vesicles in the Correze form of *Phytophthora cambivora* corresponded to its almost constant sterility.

Sporangia and zoospores were produced only when the *Phytophthora* was grown in running water. The cultures were started on carrot slants or oatmeal agar and then transferred to running water. A piece of the agar culture was cut from the dish and tied in cheesecloth before it was put in water. Even with this method the appearance of sporangia was erratic; sometimes they developed quickly and at others not at all.

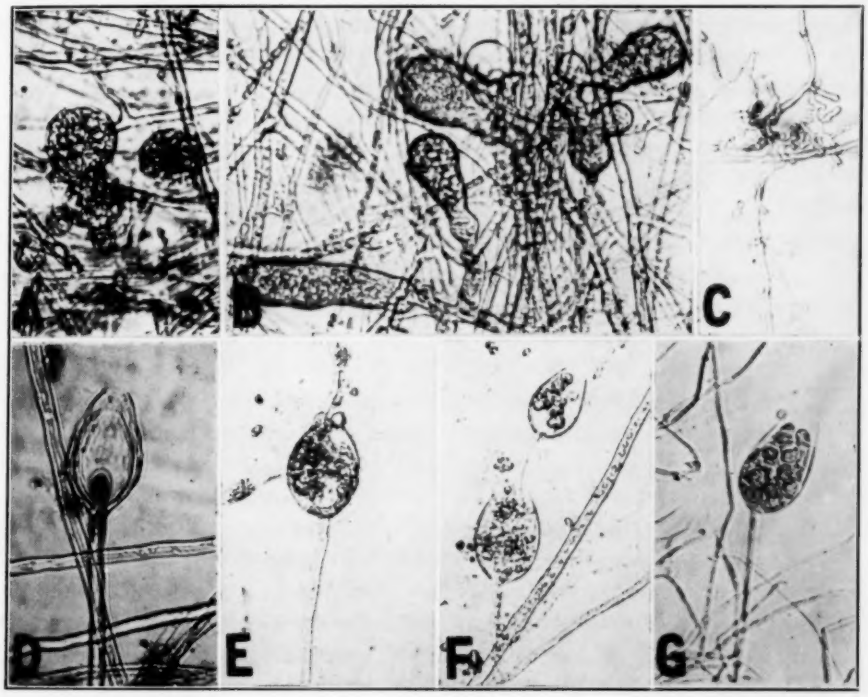

FIG. 3. *Phytophthora cinnamomi* Rands from chestnut: A. Chlamydospore-like bodies. B. Production of vesicles in sterile tap water. C. Typical hyphae with swollen tips growing on corn-meal agar. D. New sporangium forming inside emptied sporangium. E. Hypha growing from apex of sporangium. F. New sporangium produced on sporangiophore arising from base of old sporangium. G. Sporangium and zoospores.

Slightly papillate sporangia without distinct plugs were produced at the ends of long, unbranched sporangiophores and averaged 55.0×38.8 μ. Other reported sporangia sizes for *Phytophthora cinnamomi* have been 57×33 μ (30), 43.6×25.9 μ, and 46.1×31.3 μ (32). *P. cambivora* has been reported as having sporangia 47.6×31.1 μ (32), 37.4×23.7 μ (21), and 60–75 × 40–54 μ (27). The contents of the sporangium generally differentiated into zoospores (Fig. 3, G). Just before the mouth opened to liberate them there was considerable motion inside the sporangium; then an opening appeared

at the apex, and the zoospores squeezed out. They swam around vigorously for a time before coming to rest, and initiated growth by producing a single unbranched hypha. In some cases the contents of the sporangium escaped without first becoming differentiated and seemed simply to disintegrate. At other times the sporangium germinated without spore production, a hypha growing out from the apex (Fig. 3, E).

After a sporangium emptied, another usually formed inside the old case (Fig. 3, D); sometimes there were as many as four old cases outside a young developing sporangium. Often, a sporangiophore grew out of the base of an emptied sporangium and through the mouth, elongated, and gave rise to a new sporangium (Fig. 3, F). Very rarely a sporangiophore was observed growing out from immediately below an evacuated sporangium.

Since a number of persons who have worked with *Phytophthora spp.*, among whom are Leonian (20); McRae (22), and Narasimhan (25), believe them to be heterothallic, isolates from various localities in Georgia, Tennessee, and South Carolina were grown together on the chance that they might represent different segregates. Seven of them, paired in all possible combinations, were grown on oatmeal, corn-meal, potato-dextrose, and malt agars, and in Petri's solution, tap water, and distilled water, but no oogonia resulted. Furthermore, no oogonia were found under any conditions under which the isolates were studied.

Comparison with Phytophthora cambivora

The presence of both *Phytophthora cinnamomi* and *P. cambivora* in the United States makes a comparison between these species of interest. At the time of the study *P. cambivora* had not yet been found in this country and, therefore, no isolates of this species from the United States were included in the tests. Three isolates of *P. cambivora* from chestnut in Europe were compared with a number of isolates made by the writers and with 8 of *P. cinnamomi* from the following sources: 3 of Rands' from cinnamon in the East Indies, 1 of Petri's from chestnut in Europe, 1 of Cookson's from walnut in Australia, 1 of Sideris' from pineapple in Hawaii, 1 of Mehrlich's from Hawaii, 1 of White's from rhododendron. Each isolate was not used in every comparison.

No differences between isolates of the chestnut *P. cinnamomi*, greater than any of those between the other isolates, were noted; therefore, all isolates of *P. cinnamomi* are considered as a group.

Isolates of *Phytophthora cinnamomi* and *P. cambivora* were pathogenic to apple and nonpathogenic to eggplant. Those of *P. cinnamomi* were pathogenic to potato and green tomato, while those of *P. cambivora* were nonpathogenic. These results are in accord with Tucker's findings (32) on the same hosts.

No significant macroscopic differences were apparent when the isolates of *Phytophthora cinnamomi* and *P. cambivora* were grown on oatmeal, corn-meal, or malt agars. Growth on malt agar was very irregular for all strains.

On all these media, the isolates of *Phytophthora cinnamomi* were characterized microscopically by the presence of irregularly swollen hyphal tips and grape-like bunches of chlamydospores or chlamydospore-like bodies. No such growth was present in the *P. cambivora* isolates. Dark chlamydospores were present in cultures of *P. cinnamomi* on KNO_3 media; none were present in cultures of *P. cambivora* on the same media.

Optimum temperatures for growth were about 25° C. for *Phytophthora cinnamomi* and about 30° C. for *P. cambivora*. White (38) reported 25–27.5° C. for *P. cinnamomi*, while Tucker (32) reported 25–27° C. for *P. cinnamomi* and 27–30° C. for *P. cambivora*.

Leonian (19) found considerable difference in reaction to the presence of malachite green in various concentrations in nutrient solution. He reported sporadic growth of *Phytophthora cambivora* at $\frac{1}{16}$ and $\frac{1}{8}$ ppm. of dye and no growth at $\frac{1}{4}$ ppm., whereas *P. cinnamomi* grew sporadically at $\frac{1}{4}$ ppm. of dye. In our tests isolates of *P. cambivora* made some growth at $\frac{1}{16}$ ppm. of dye and none in stronger solutions, whereas isolates of *P. cinnamomi* showed slight growth in concentrations up to and including $\frac{1}{2}$ ppm. of dye.

No oogonia were ever found in the writers' isolates of *Phytophthora cinnamomi*, although cultures were grown in comparison with other isolates of *P. cinnamomi* and *P. cambivora* on media suggested by previous workers: water agar (1), synthetic agar (1), KNO_3 (32), soil agar (5), grated-carrot agar (17), and carrot-extract agar (28). Oogonia have been reported in old cultures of *P. cinnamomi* by Ashby (2) and on KNO_3 by Tucker (32). Ashby (2) has reported them in old cultures of *P. cambivora*, while Petri (28) found them in this species on carrot-extract agar.

INOCULATIONS

Potted plants in the greenhouse were inoculated; the majority with aerial hyphae produced on oatmeal agar. Wound inoculations were generally made on the main root, a short distance below the collar. In tests without wounds, the inoculum, agar cultures with as little agar attached as possible, was planted in the soil. Results obtained from this type of inoculation indicate that wounding did not increase the percentage of infection.

When infested soil was the inoculum source, the plants were removed from soil in which they were growing and replanted in soil in which other inoculated plants had previously died. The *Phytophthora* was able to live at least 1 or 2 months saprophytically in the soil and to kill plants transplanted into it.

Representatives of 5 tree genera were inoculated. The oaks and oak-like *Lithocarpus* were included because of their close relationship to *Castanea*, and walnut and beech because *P. cambivora* had been reported on them (9, 10).

As shown in table 1 the European and American species of *Castanea* were much more susceptible than the Oriental species to isolates of the chestnut *Phytophthora cinnamomi*. The percentage figures for susceptibility are in

most cases the average of the results of inoculations with several different isolates of *P. cinnamomi* from chestnut. Some of the isolates killed 100 per cent of the American species of *Castanea* while others were comparatively weak. Comparisons between isolates are further discussed under the nursery phase of the root-rot problem. It is interesting that in Europe *C. mollissima* and *C. crenata* have also been reported as resistant to *P. cambivora* (14). In

TABLE 1.—*Susceptibility of various tree species to isolates of Phytophthora cinnamomi from chestnut and chinkapin*

Species	Plants inoculated	Killed by *Phytophthora*	Check plants	Dead[a]
	No.	*Per cent*	*No.*	*Per cent*
Castanea alabamensis Ashe	140	70	30	57
C. alnifolia Nutt.	113	82	30	57
C. ashei Sudw.	16	94	4	0
C. crenata Sieb. and Zucc.	227	4	101	1
C. dentata (Marsh.) Borkh.	86	70	54	2
C. henryi (Skan) Rehd. and Wils.	81	2	50	0
C. margaretta var. *arcuata* Ashe	10	60	2	0
C. mollissima Blume	197	1	102	0
C. ozarkensis Ashe	60	70	12	25
C. pumila (L.) Mill.	107	74	30	23
C. sativa Mill.	251	38	124	2
C. seguinii Dode	8	0	4	0
Fagus grandifolia Ehrh.	31	0	24	0
F. sylvatica L.	40	0	5	0
Juglans nigra L.	59	0[b]	36	0
Lithocarpus cuspidatus (Thunb.) Nakai	3	0	3	0
L. densiflorus (H. and A.) Rehd.	6	50	8	0
Quercus alba L.	75	3	57	0
Q. agrifolia Née	10	0	5	0
Q. borealis Michx. f.	56	4	28	0
Q. coccinea Muench.	13	0	13	0
Q. garryana Dougl.	54	0	30	0
Q. macrocarpa Michx.	18	0	12	0
Q. marilandica Muench.	21	0	10	0
Q. montana Willd.[c]	157	1	80	0
Q. palustris Muench.	69	0	37	0
Q. phellos L.	25	0	12	0
Q. prinus L.	13	0	11	0
Q. rubra L.	38	0	37	0
Q. velutina Lam.	29	0	15	0

[a] *Phytophthora* could not be isolated from any of the dead check plants.

[b] In later experiments isolates from *J. regia* L. and *J. nigra* L. were able to kill 40 per cent of 5-year-old trees, while a virulent strain from red pine caused no root rot.

[c] Called *Q. prinus* (24).

a laboratory test *C. dentata* was susceptible to one of the European isolates of *P. cambivora*.

The 3 species of *Quercus* that were infected artificially with *Phytophthora cinnamomi* were later found naturally infected in nurseries (6). Neither American beech (*Fagus grandifolia*) nor European beech (*F. sylvatica*) became infected. This is an interesting difference between *P. cinnamomi* and *P. cambivora*, since in England (11) *P. cambivora* has been found causing root rot on European beech, and *P. cinnamomi* has not been reported from this host.

Black walnut was not infected, under the conditions of the experiment, with any of the isolates of *P. cinnamomi* from chestnut. Later these same trees were successfully inoculated with isolates from root rot of seedling black walnuts (*Juglans nigra* L.) and Persian walnuts (*J. regia* L.).

Some years ago, Dr. N. I. Vavilov, of the Institute of Plant Industry, Leningrad, U.S.S.R., visited the chestnut breeding and disease plots of the Bureau of Plant Industry near Washington and was considerably interested in the possible susceptibility of the Russian chestnuts, which grow so extensively in the Caucasian Mountains, to this root disease and to the chestnut blight. Arrangements were made for an exchange of seed, whereby Dr. Vavilov sent seed to the United States in 1933 from the Russian chestnut trees and the Division of Forest Pathology sent him seed of some of its more promising hybrids to test against Russian diseases.

Inoculations with *Phytophthora cinnamomi* showed that the Russian chestnuts were fully as susceptible to the root disease as were the other strains of the European chestnut that this Division tested. The Russian chestnuts from the Caucasian Mountains were also moderately susceptible to the chestnut blight disease, caused by *Endothia parasitica*. No information has been received from Dr. Vavilov for some time about the reactions of our chestnuts to the Russian diseases.

FIELD TESTS OF ASIATIC CHESTNUTS

Test plantings of Asiatic chestnuts have been established on soils in areas where the root disease is active on native *Castanea* or where it has appeared on nursery stock or in orchards. These plantings were in Alabama, Arkansas, Georgia, Maryland, Mississippi, Louisiana, and Tennessee. From these tests it has been determined that all available strains or selections of *Castanea crenata, C. mollissima, C. henryi* (Skan) Rehd. and Wils., and *C. seguinii* Dode have a high degree of resistance to *Phytophthora cinnamomi,* which causes only small localized lesions on the roots. Individuals of *C. sativa,* known to be susceptible to root rot, were placed in the plantings to act as checks. These trees died within a year or two. Resistant trees remained in the plots from 5 to 11 years and were unaffected by the root disease. In two isolated cases where seedlings of *Castanea crenata* and *C. mollissima* were growing in waterlogged soils, losses from root rot have occurred, but how much damage resulted from water and how much from fungus was undecided.

PHYTOPHTHORA ROOT ROT OF NURSERY STOCK

Hosts

In 1933, following early spring floods, heavy losses were experienced in beds of 3-year-old red pine (*Pinus resinosa*) in a Maryland nursery. The disease was first thought to be of physiological origin because of the failure to isolate a pathogen by any recognized technique. However, the disease continued to spread notwithstanding the return of more or less normal conditions in the nursery. With the water-blank method described previously

a *Phytophthora,* later found to be *P. cinnamomi,* was isolated and its pathogenicity was proved (16).

For several years losses, often of epidemic proportions on the more susceptible species,[4] occurred in seedbeds and plantings of various tree species in the nursery where this disease was first noticed and in other nurseries as follows: *Pinus resinosa* from 2 nurseries in Maryland and 1 in Delaware; *P. sylvestris* L. from 1 nursery each in Maryland and Virginia; *P. strobus* L. from 1 Maryland nursery; *Taxus cuspidata* Sieb. and Zucc.* from 3 nurseries in Maryland and 1 in Virginia; *T. baccata* L.* and *T. media* Rehd.* from 1 nursery in Maryland; *Picea abies* Mill. from 1 nursery each in Maryland and Virginia; *P. pungens* Engelm. from 1 nursery in Maryland; *Larix decidua* Mill.* and *L. leptolepis* (Sieb. and Zucc.) Gord.* from 1 Maryland nursery; *Juglans nigra** from 2 Maryland nurseries and 1 each in North Carolina, Arkansas, and Louisiana; *J. regia** from 1 Maryland nursery; *Quercus borealis** and *Q. montana* from 2 Maryland nurseries; and the following each from 1 nursery in Maryland: *Rhododendron mucronulatum* Turcz.,* *Betula alba* L., *B. papyrifera* Marsh.,* *Platanus orientalis* L., *Robinia pseudoacacia* L., and *Quercus alba.*

Symptoms

On both coniferous and broadleaf species the fungus commonly causes a root rot of seedlings and transplants. While apparently uncommon, an instance is known in a single nursery where *Phytophthora cinnamomi* initially produced typical damping-off. On seedlings, the above-ground symptoms of the typical root rot are first noticed in the late spring or summer. The first indication of trouble is the loss of the normal green of the foliage, followed by wilting in the broadleaf species and by dieback and less obvious wilting in the coniferous species. On older nursery stock the symptoms in the above-ground portions usually do not appear until the root system is almost completely rotted. The tree, therefore, appears to decline rapidly, with death soon following. Leaf symptoms on such trees often appear after the causal organism has become inactive and are not uncommonly attributed to other causes, such as drouth or twig blight.

On coniferous stock beyond the damping-off age, the fungus causes a dry type of root rot accompanied by resin deposition on and in the infected portions, including the wood. This infiltration of the wood at the point of attack is particularly valuable as a distinctive symptom, since fungi that kill bark and cause external resin flow usually do not cause resin deposition in the xylem. The areas invaded by the fungus are reddish-brown. The very recently invaded tissue is characterisitcally mottled brown and light green, except in species of *Taxus* where recently infected tissue is lightly streaked with black.

On broadleaf species the rot produced is dry, except in the case of *Robinia* and *Juglans.* Members of these two genera also differ in that the color of

[4] Indicated by asterisk.

the rot is not typical. The rot produced on *Robinia* is the same color as the healthy surrounding tissue, while on *Juglans* the rot is black. In all other species seen, the rot is reddish-brown, and, like that on the conifers, is mottled brown and light green in recently invaded tissue.

In both coniferous and broadleaf species no definite line of demarcation is found between the invaded and healthy tissue. The fungus advances into sound tissue in irregular wedge-shaped streaks. *Phytophthora cinnamomi* apparently is capable of direct penetration into healthy cells and was not found in cultures made far behind the zone of active penetration. Data taken from an inoculation experiment on ninety 3-year-old *Pinus resinosa* give an indication of its rate of spread within the host. Thirty-two days after inoculation 61 per cent of the population was dead. The average spread of the fungus recorded at the time girdling and death of these trees occurred was 9 cm. up and 5 cm. down from the point of inoculation, or 2.8 mm. up and 1.5 mm. down per day. The fastest spread of the fungus during this period was 14 cm. up and 5 cm. down from the inoculation point, or 4.3 mm. up and 1.5 mm. down per day.

Phytophthora cinnamomi apparently often attacks just below the soil line and frequently occupies a portion of the stem of infected trees. The presence of the fungus in tissue above the soil line is often indicated, in conifers, by external resin flow, and, in broadleaves, by a depressed area above the dead cambium.

In the United States, except in the case of *Juglans nigra*, no exactly similar diseases of the hosts, caused by other fungi, have been described. A disease of walnut identical in symptoms but caused by another member of the same genus, *Phytophthora cactorum*, has been reported (8). It can be distinguished from *P. cinnamomi* root rot only by cultural methods. As yet, the fungi have not been found together on walnut, although both have been found in the same nursery. They have, however, been found together in the same seedling in cases of late damping-off of red pine.

A root disease of *Pinus resinosa*, differing somewhat in symptoms, has been found in the same nursery and occasionally on the same tree as the Phytophthora root rot. The symptoms of this disease, caused by *Sphaeropsis ellisii* Ell. and Ev. (7), differ from those produced by the *Phytophthora* in that the infected tissue is deeper red with black streaks running through it and into the xylem. No resin deposition is associated with this rot.

Inoculations

Inoculation tests with *Phytophthora cinnamomi*, using four to fifteen 2- to 5-year-old vigorously growing potted trees, with re-isolation of the pathogen, agree with the field observations that *Pinus resinosa*, *Larix decidua*, and *Juglans nigra* are highly susceptible. *J. nigra* was susceptible to isolates from *J. nigra* and *J. regia* in Maryland, but not susceptible to isolates from *P. resinosa* in Maryland or *Castanea dentata* in Georgia. It was not possible to apply Koch's rules of proof to a number of the individual

species from which *P. cinnamomi* was isolated, because they were not available for inoculation. Twenty isolates from 16 different hosts growing in seven states have, however, all proved to be identical in that they can produce the root disease on *P. resinosa* and *C. sativa* and can be reisolated from such cases of rot. Species such as *Picea abies* and *Robinia pseudoacacia,* found only slightly susceptible in the field, were completely resistant in the inoculation tests. As a result of the greenhouse inoculations Douglas-fir (*Pseudotsuga taxifolia* (Poir.) Britton) has been found highly susceptible to root rot caused by isolates from *C. pumila* in South Carolina, *J. nigra* and *P. resinosa* in Maryland, and *Taxus cuspidata* in Virginia. *Fagus grandifolia* and *F. sylvatica* were not susceptible to isolates from Maryland, Delaware, Virginia, and Georgia. *P. mugo* var. *mughus* (Scop.) Zenari, tested because it is grown as an ornamental associated with yews and often is used to replace yews that die of root rot, was not susceptible to any of the isolates. No root rot has ever been found on this species in nurseries or plantings. The results of the cross inoculations indicate that there are probably a number of slightly different physiologic strains of *P. cinnamomi.* In a small laboratory test, one of Rands' original isolates (30) of *P. cinnamomi* from *Cinnamomum burmanni* Nees caused root rot of *Pinus resinosa* that was indistinguishable from that caused by isolates from that host. The isolate was, however, only weakly parasitic, as compared with isolates from red pine, since it took about 4 times as long to invade an equivalent amount of tissue.

Conditions Predisposing to Attack

As previously mentioned, the first observed loss on *Pinus resinosa* was an epidemic apparently induced by abnormally wet soil conditions. Subsequent losses of this and other tree species have been sporadic. Generally such losses have been heavy. In the instances where a period of abnormally wet weather was not the apparent predisposing factor, the trees involved usually had been planted on a poorly drained site. While conditions of poor soil aeration create a favorable condition for attack by the fungus, when a susceptible host is invaded the fungus apparently is capable of killing it regardless of soil or weather conditions.

An experiment was made to determine the effects of soil and water on the incidence of the disease, with 3-year-old seedlings of *Pinus resinosa* taken from beds in which the disease was present. The trees selected apparently were free from root rot, but no attempt was made to wash or sterilize the roots. Thirty trees were potted in greenhouse soil and an additional 30 in a mixture of half soil and half sand. One third of each group were given only enough water to keep them alive, another third 3 times as much, while the remaining third were watered 6 times as much. At the end of 118 days, in the groups given the largest amount of water, 8 seedlings in soil and 4 in the soil-sand mixture were dead; of the group given 3 times the minimum watering, 4 in the soil and 2 in the soil-sand mixture were dead; while of those given the minimum amount of water, 3 in the soil and 2 in the soil-sand

mixture were dead. *Phytophthora cinnamomi* and in some of the trees *Sphaeropsis ellisii* as well were isolated from the dead trees. Wager (35) was able to artificially infect avocados with *P. cinnamomi* when pots in which they were growing were submerged in water for periods as short as 2 or 3 days. Infection was not possible with trees given normal watering.

Phytophthora cinnamomi has been isolated from root-diseased nursery stock growing in soils ranging in pH from 3.2 to 7.0. In a single experiment, in which each of 20 pots, with soil adjusted to pH values of 4.2, 5.0, 7.0, and 8.0, were planted with 1 inoculated and 1 healthy *Pinus resinosa* tree, results were inconclusive since an equal number of the healthy trees under each pH value contracted the disease. The finding of the fungus causing root rot on nursery stock growing in soils with a considerable pH range would seem to indicate that while the fungus may be affected by an adverse pH, as has been shown by White (38), it is nonetheless able to grow and infect susceptible hosts. Once infected, the effect of pH probably would be more important as it affected the vigor of the host itself, rather than the fungus. Field tests to control root rot in seedbeds of red pine and blue spruce by adjusting the acidity with sulphur and aluminum sulphate were unsuccessful. The fungus continued its activities at pH readings as low as 3.3, at which point the host was injured by the acid condition.

CONTROL

No satisfactory control measures for this disease in the nursery have been developed. When rot is observed on stock being transplanted, all infected individuals should be isolated, and preferably destroyed. At least they should not be planted near healthy susceptible species. In nursery soil known to be infested with *Phytophthora cinnamomi,* poorly drained sites and sites on which losses have occurred should be avoided or adequate draining should be provided when planting susceptible species. Care should be taken to avoid overdense stands in the seedbeds of susceptible species.

All sanitation practices that tend to stop movement of infested soil and plant material within the nursery should be used. Such soil and plant material should never be added to a compost heap. For such species as *Juglans nigra,* which can be planted directly in the forest, seedbed planting in areas where losses from *Phytophthora cinnamomi* have occurred should be held to a minimum.

Before work on this disease was started, blight had already appeared on American chestnuts in the areas where Phytophthora root rot was found. Absence of any effective control for blight, in either orchard or forest chestnuts, made control of root rot immaterial. Asiatic chestnuts, brought to the United States because of their blight-resistant qualities, are also resistant to root rot. Hybrid varieties, resistant to both blight and root rot, are being developed by crossing American chestnut and chinkapins with Asiatic species. In Spain, Urquijo (34) reports control of root rot, presumably caused by *P. cambivora,* by removing the soil from the collar region of infected trees, cleaning the affected area after the fungus is presumably killed by

desiccation, applying a sticker, and then dusting with copper carbonate. This protection is reported to last for a number of years after the soil is refilled around the collar region. The majority of root-diseased chestnuts examined in the southeastern United States usually had most of the main lateral roots infected as well as the collar region. Often death occurred because most of these lateral roots were girdled without the collar region becoming involved. However, it appears that the control described might be practical if the host tree involved was less susceptible than the American chestnut. Varieties of the European chestnut are in orchard plantings in the United States, and our tests show them to be less susceptible than the American chestnut to *P. cinnamomi*. In blight-free areas top working of root-rot susceptible varieties on resistant Asiatic root stock offers an obvious control method.

SUMMARY

Phytophthora cinnamomi Rands has been found causing root rot, similar to the "ink disease" of chestnut in Europe, on the American chestnut in Maryland, Virginia, North and South Carolina, Georgia, Alabama, Tennessee, and Mississippi. It has been found causing the same disease on *Castanea ozarkensis* in Arkansas. Other native chinkapins have been killed by the *Phytophthora* where they occurred within the range of the chestnut. It seems probable that this disease is responsible for the recession of the American chestnut from the lower Piedmont area of the Atlantic States into the higher elevations of the mountains and from southern Mississippi and Alabama into the hilly regions in the northern parts of those States. It is known to have caused the recession of the Ozark chinkapin. The pathogenicity of *P. cinnamomi* has been demonstrated on its native chestnut and chinkapin hosts.

In addition, this fungus is the cause of a similar root disease on 20 coniferous and broadleaf hosts growing in nurseries in Arkansas, Delaware, Georgia, Louisiana, North Carolina, Maryland, and Virginia.

Field and greenhouse inoculation tests have demonstrated that the European chestnut, *Castanea sativa*, is susceptible to the disease, while the Asiatic species, *C. crenata*, *C. mollissima*, *C. henryi*, and *C. seguinii*, have a high degree of resistance to it.

In greenhouse inoculation tests, Douglas-fir (*Pseudotsuga taxifolia*) was highly susceptible to the root rot *Phytophthora*.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY,
SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MD.,
IN COOPERATION WITH SCHOOL OF FORESTRY, UNIVERSITY OF GEORGIA,
ATHENS, GEORGIA.

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BLIGHT OF ORIENTAL ARBORVITAE

A. G. PLAKIDAS

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Several varieties of the oriental arborvitae (*Thuja orientalis* L.) are extensively grown in Louisiana and other parts of the South as ornamental plants. For several years[1] these have been subject to a very destructive disease known locally as "blight" or "fire." The two varieties most commonly grown in Louisiana, Berckman's Golden and Baker, are very susceptible. Another variety, the Rosedale, which was once very popular, is virtually out of existence at present because of this disease. The same trouble, apparently, affects also the Italian cypress (*Cupressus sempervirens* L.). The symptoms on this host are similar to those on arborvitae, and the fungus proven experimentally to be the cause of the blight on arborvitae appears, morphologically and culturally, identical with that on diseased Italian cypress. However, no cross-inoculation tests have been made.

The exact geographical distribution of the disease is not known. It is definitely known that, besides Louisiana, it is present in the neighboring states of Texas, Mississippi, and Arkansas, and it is presumed to occur in other parts of the South where arborvitae are grown.

In the past the disease has been variously attributed to red spider, drought, excessive summer heat, or winter injury. A critical study during the past two years has shown definitely that the disease is caused by a fungus, an apparently undescribed species of *Cercospora* (6). Because of the importance of the disease, and because preliminary experiments have shown that it is amenable to control with copper sprays, the results of the investigation are presented at this time with the hope that they may be of interest to other investigators and of practical use to nurserymen and others. Work is in progress to determine the host range of the fungus, the period during which infection takes place, and the most practical method of control.

DESCRIPTION OF THE DISEASE

Affected leaves and branchlets are killed, turn brown, and fall off gradually. The shedding of the branchlets leaves the plant thin and ragged (Figs. 1 and 2, A). Approximately the lower two-thirds of the plant is the most severely affected. Usually there is a tuft of healthy growth at the top of even severely diseased plants. Very often, one side of the plant will be practically killed with only scattered dead areas on the other side. This is particularly true of plants growing close to houses: the side away from the wall is usually more severely diseased than the one next to the wall. The disease is more severe in nurseries than in gardens, probably because the plants in the nursery are close together and the fungus spreads easily from

[1] No authentic records regarding the earliest appearance of the disease have been found, but from talks with nurserymen and others, it was learned that blight has been known probably for 20 to 25 years.

plant to plant. In the nursery row, infected plants are often killed in a relatively short time (1–3 years); in gardens, affected plants may persist in a ragged, unhealthy condition almost indefinitely.

Cankers also have been observed on young stems. These girdle the bark and kill the small twigs. The portion of the stem immediately above the ringed bark swells considerably (Fig. 2, B) giving the appearance of an insect gall. Stem cankers, thus far, have been found only on one variety,

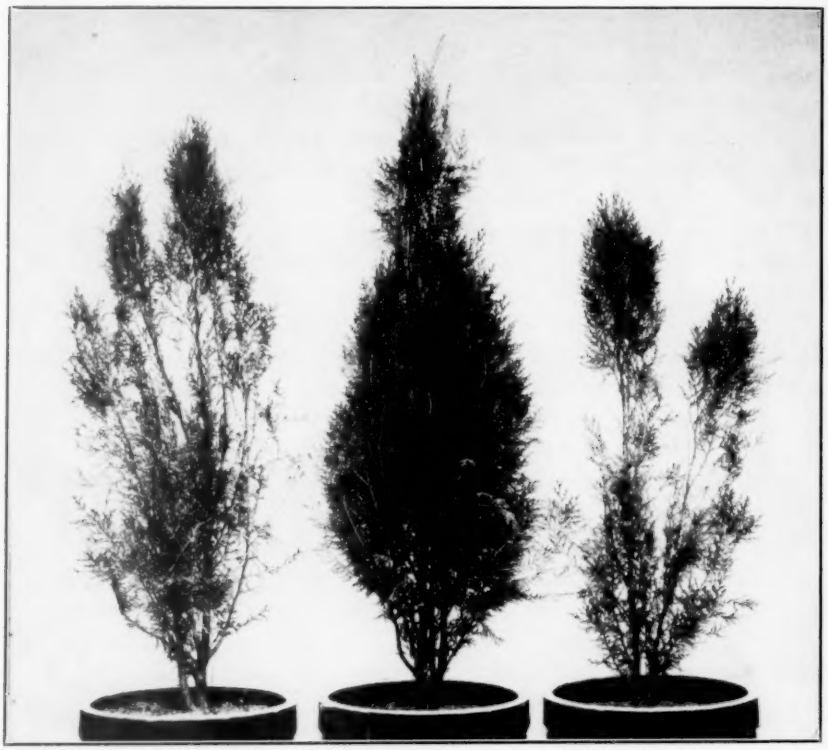

FIG. 1. Contrast between check (center) and inoculated arborvitae, variety Baker. The plants were inoculated with pure cultures of *Cercospora thujina* July 14, 1943, and photographed May 20, 1944. The illustration shows the thin, ragged condition of the diseased plants which results from the shedding of the diseased branchlets, but does not show the brown color of the dead foliage. ×0.1.

Berckman's Golden. The same fungus that causes the leaf blight has been isolated from the stem cankers.

CAUSE OF THE DISEASE

Isolations. A fungus, apparently a *Cercospora*, has been constantly associated with the disease. The fungus was first isolated from diseased material of the Baker variety collected in a nursery in Folsom, Louisiana, April 6, 1943. Since then it has been found on specimens of diseased arborvitae collected from different parts of Louisiana and on one specimen re-

ceived from Arkansas. Cultures of the fungus have been obtained both by planting pieces of diseased leaves on agar and by isolating single conidia.

In addition to the *Cercospora*, perithecia of *Mycosphaerella sp.* were often found on diseased material, either alone or in combination with *Cercospora*. The *Mycosphaerella* was obtained in culture by isolating single ascospores. As isolates of the two fungi appeared similar in culture, it was at first assumed that the *Mycosphaerella* represented the ascigerous stage of

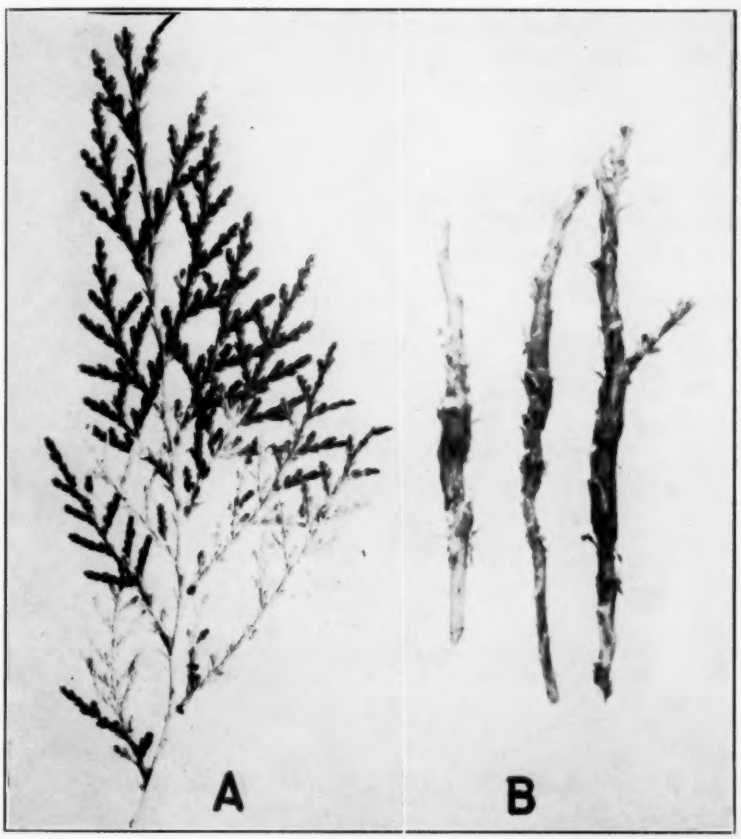

FIG. 2. A. Arborvitae (var. Baker) twig showing difference in color between healthy and diseased foliage and branchlets. B. Bark cankers on stems of Berckman's Golden variety. Both approx. natural size.

Cercospora. However, the *Mycosphaerella* proved non-pathogenic to arborvitae (Table 1), and it must be assumed that it has no connection with the *Cercospora*.

Pathogenicity. All the inoculations were made on healthy arborvitae, variety Baker, about 3 feet in size and planted in 4-gallon crocks. As the fungi used in these experiments did not sporulate in culture,[2] the inoculum

[2] Of a total of 29 isolates of *Mycosphaerella*, two produced a few conidia in culture. These varied from one-celled elliptical to 2-septate fusiform-cylindrical. None of the 54 isolates of *Cercospora* sporulated in culture.

was prepared by macerating the mycelium, together with the agar substratum, in water by means of a mechanical blender according to the method suggested by Andrus (1). Young cultures, 9–12 days old, grown on potato-dextrose agar, were used to make the inoculum, which was sprayed on the plants by means of a small insect sprayer. The inoculated plants were placed under large bell jars for periods ranging from 48 to 96 hours, then removed and placed outdoors. Inoculations were made also by placing diseased, conidia-bearing twigs on healthy plants.

The results of the inoculation experiments are in table 1. Infection with *Cercospora* was obtained in every case. On plants inoculated during the summer (July 14 to September 20) infection was heavy, approximately 50 to 75 per cent of the foliage becoming necrotic by the end of 5 to 6 weeks after inoculation. The period lapsing between inoculation and the appear-

TABLE 1.—*Summary of inoculation experiments on Thuja orientalis L., var. Baker, with Cercospora thujina and Mycosphaerella sp.*

Treatment	Time of treatment[a]	No. plants	
		Inoculated	Diseased
Cercospora thujina			
Pure culture	Summer	8	8
	Winter	4	4
Conidia-bearing twigs	July 14 and Aug. 4, 1943	3	3
Mycosphaerella sp.			
Pure culture	Summer	7	0
Check			
Not treated	Summer	71	0
Atomized with H_2O, placed in moist chamber	Summer	4	0
	Winter	3	0

[a] Summer inoculations were made on July 14, Aug. 4 and 14, Sept. 2, 9, and 20, 1943; winter inoculations were made on Jan. 6, Feb. 8, and Mar. 17, 1944.

ance of disease symptoms varied considerably in the different experiments. In the first inoculation made on July 14, the early symptoms (yellowish discoloration of the leaves) appeared in 2 weeks, and necrotic lesions in 4 weeks after inoculation. In all other inoculations made during the summer, first evidence of infection was apparent about 4 weeks after inoculation and typical necrotic lesions in 5 to 6 weeks.

With inoculations made during the winter (January 6, February 8, and March 17), an entirely different situation was encountered. The inoculated plants were examined once a week until April 25, and, except for a trace of disease on one small twig of one of the two plants inoculated on January 6, they remained apparently healthy. It was concluded then that the results of the winter inoculations were negative and that infection apparently did not occur during the cool season. However, when the plants were reexamined on May 15, all were typically diseased, although the infection was much lighter than on the plants inoculated during the summer. It is not

known whether infection occurred at the time of inoculation and the disease did not develop during the cool season, or whether the fungus remained viable externally on the leaves and produced infection with the advent of warm weather. This question is important from the control point of view. Development and spread of the fungus in natural infections have not been noted during the winter, although viable conidia have been found on diseased plants during the winter months.

The symptoms produced by inoculation were typical of the disease as it occurs naturally. The fungus sporulated abundantly on the inoculated plants. It was reisolated many times, and it produced the disease when reinoculated into healthy plants.

The *Mycosphaerella sp.* (29 isolates) was used to inoculate 7 plants on 6 different dates (Table 1), and in not a single case did it produce infection. It must be concluded, therefore, that it has no connection either with the disease or with the *Cercospora* which is the cause of the disease.

THE FUNGUS

Identity

No report of *Cercospora* on *Thuja* has been found in literature, although this genus has been reported on related conifers. Ellis and Everhart (2) reported *C. Sequoiae* on *Sequoia gigantea,* and *C. Sequoiae* var. *Juniperi* on *Juniperus virginiana.* Satisfactory specimens of the type material of these were not seen by the writer. A small fragment of Ellis and Everhart's type specimen of *C. Sequoiae* on *Sequoia gigantea* was obtained from the Farlow Herbarium through the kindness of Dr. D. H. Linder, but neither sporophores nor conidia were found on it.

Three specimens (2 herbarium and 1 fresh) of *Cercospora Sequoiae* var. *Juniperi,* all on *Juniperus communis* var. *depressa,* were examined. One, obtained from the Farlow Herbarium, was collected by J. J. Davis in Melvina, Wisconsin, June 12, 1916. The second was obtained from Dr. Charles Chupp, of Cornell University, and was collected by H. C. Green in Madison, Wisconsin, June 10, 1943. The third, which was fresh, was received directly from H. C. Green and was collected in the same locality on August 16, 1943. Since the original description of *C. Sequoiae* var. *Juniperi* by Ellis and Everhart was based on the specimen collected by J. J. Davis in Wisconsin, it is reasonably safe to assume that Davis' determination of the fungus on *J. communis* var. *depressa* was accurate, and, therefore, that the Wisconsin specimens examined by the writer actually represented *C. Sequoiae* var. *Juniperi* E. and E.

The same fungus was found on all three specimens. It was studied rather critically in comparison with the fungus occurring on arborvitae, and it was concluded that the two are entirely distinct. The juniper fungus does not appear to be a *Cercospora,* and, in fact, it appears doubtful that it belongs even in the same family with *Cercospora.* Its fruiting body (Fig. 3, D) is composed of a thick sclerotial stroma which extends deeply into the

parenchyma tissue and becomes erumpent, forming a raised, cushion-like structure on the surface of the leaf from which arise very numerous short conidiophores. The fruiting body is definitely a sporodochium; the fungus is probably an *Exosporium*. Other differences were noted. The conidia and conidiophores of the juniper fungus are decidedly shorter than those of the arborvitae fungus. The juniper fungus was obtained in culture by isolating single conidia and compared with cultures of the arborvitae fungus. Cultures of the two fungi on the same medium were distinctly different.

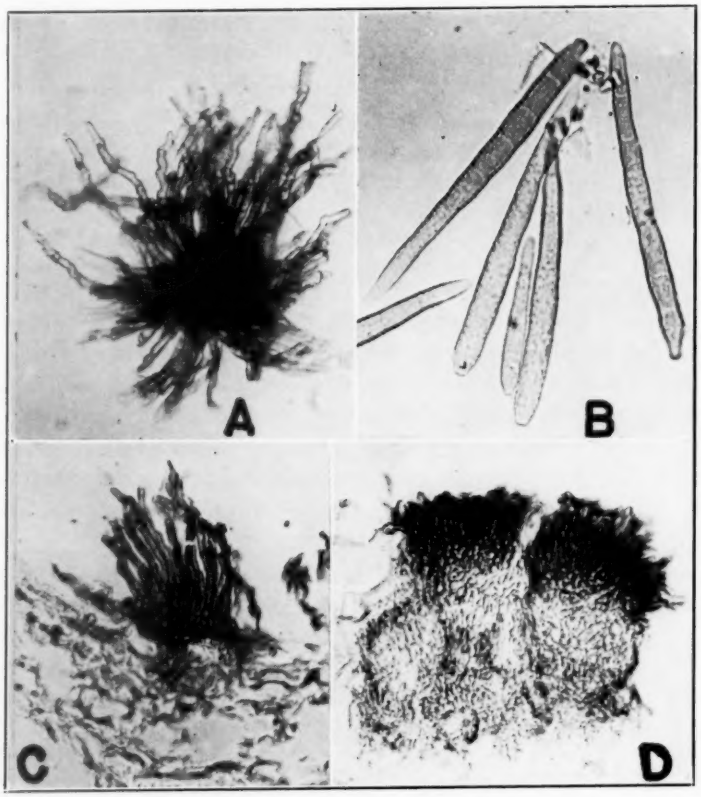

FIG. 3. A. *Cercospora thujina*. Sporophore fascicle pressed between glass cover slips. ×180. B. *Cercospora thujina* conidia greatly enlarged to show the echinulation. ×780. C. Section through fructification of *C. thujina* showing that the conidiophores arise from a thin stromatic base (compare with D). ×180. D. Section through fructification of *Cercospora Sequoiae* var. *Juniperi* showing the thick, sporodochium-like sclerotial stroma from which arise short conidiophores. ×180.

The arborvitae fungus agrees somewhat with the descriptions of *Cercospora Sequoiae* in respect to the size, shape, and color of its conidiophores and conidia. However, the following statement in Ellis and Everhart's description of *C. Sequoiae* suggests that the fruiting body of this fungus, notwithstanding its longer conidiophores, is probably similar to that of *C. Sequoiae* var. *Juniperi:* "Forming large, compact, olivaceous tufts which, under lens,

resemble perithecia of *Sphaerella.*" The fruiting bodies of the arborvitae fungus do not resemble perithecia when viewed under lens. They consist of fascicles of spreading conidiophores originating from a narrow base.

Georgescu and Badea (4) described *Cercospora juniperina* on *Juniperus communis* in Roumania, although in an earlier publication (3), these authors had named their fungus *Camarosporium juniperinum.* They described the conidia as echinulate. Later, Sandu-Ville (7) collected and examined specimens of the fungus described by Georgescu and Badea and reported that the fungus was neither a *Camarosporium* nor a *Cercospora* but an *Exosporium,* probably *E. deflectens* Karst.

Dr. Charles Chupp, who kindly examined specimens of the arborvitae fungus submitted to him, found the conidia echinulate like those of *C. Sequoiae* and *C. Sequoiae* var. *Juniperi* which he had examined earlier. At that time, Dr. Chupp was of the opinion that these three fungi were either identical, or very closely related, and, in a letter, expressed the opinion that they did not fit in the genus *Cercospora,* first because of the echinulation of their conidia, and secondly because of the promiscuity in their host relationship.

The arborvitae fungus is apparently distinct from those on *Sequoia* and *Juniperus.* Furthermore, it is doubtful that strict host specificity in the case of *Cercospora* has been proven unquestionably. The objection regarding the echinulations of the conidia is more valid because no provision is made in the description of the genus *Cercospora* for inclusion of forms with echinulate spores. In the case of the arborvitae fungus (also of those on *Sequoia* and *Juniperus*) the echinulations are so faint and indistinct that fairly high magnification is necessary (Fig. 3, B) to see them. In fact, Ellis and Everhart apparently failed to note the echinulation, for they made no mention of it in their description of *C. Sequoiae* and *C. Sequoiae* var. *Juniperi.* In all other characters, the arborvitae fungus fits well in the genus *Cercospora,* and it is therefore described as a new species under the name of *C. thujina,* n. sp.

Description

The fungus fructifies fairly abundantly on recently killed leaves and branchlets, producing grayish tufts of conidiophores which, when examined with a dissecting microscope, have the outline of shrubs with relatively narrow bases and spreading branches. The conidiophores (Fig. 3, A) which are very numerous in each fascicle (as many as 50 have been counted) arise from a relatively thin, more or less disc-like stromatic base. They are long, flexuous, sparingly septate, rarely branched, somewhat irregular in diameter, yellowish-brown, with several spore scars. The conidia (Fig. 3, B) are oblong, tapering, with obconic-truncate bases, of approximately the same color as the conidiophores, septate, faintly echinulate.

The fungus grows easily but slowly in culture. The colonies vary from dark greenish gray on some media (bean-pod agar for example) to light

gray on others (potato-dextrose, corn-meal and oatmeal agars). Patches (not sectors) of whitish aerial growth often develop on the colonies. Spores were never observed in culture, although many attempts were made to induce sporulation by growing the fungus under different environmental conditions on a variety of media, such as bean-pod, oatmeal, corn-meal, potato-dextrose, and Czapek agars, and on autoclaved bean pods, potato (white and sweet) plugs, and arborvitae twigs either alone or with the addition of soil to the flasks.

Cercospora thujina, n. sp.

Conidiophores in dense fascicles arising from a relatively thin, disc-like, stromatic base, long, flexuous, sparingly septate, irregular in diameter, yellowish brown, with several spore scars, 26.0–105 × 3.3–5.0 μ (average 55.8 × 4.3 μ); conidia oblong, tapering, straight or slightly curved, with obconic-truncate bases, yellowish brown, 1–7 septate (average number of septa 4.2), faintly echinulate, 19.0–56.0 × 4.9–6.6 μ (average 42.2 × 5.5 μ). Parasitic on *Thuja orientalis* L. causing necrosis of leaves and branchlets and cankers on twigs.

Type locality: Baton Rouge, Louisiana, U. S. A.

Type specimens deposited in the herbarium of the Department of Botany, Louisiana State University, under accession No. 4677, and also in the Mycological Collections, Bureau of Plant Industry, Washington, D. C., and in the Farlow Herbarium, Harvard University.

Maculis indeterminatis, brunneis; conidiophoris in fasciculis densis e basi stromatica, tenui planaque oriundis, longis, flexuosis, parce septatis, crassitudine inaequalibus, fulvis, cicatrices aliquot conidiis factas habentibus, 26.0–105 × 3.3–5.0 μ; conidiis oblongis, attenuatis, rectis vel subcurvatis, bases obconico-truncatas habentibus, fulvis, 1–7 septatis, minute echinulatis, 19.0–56.0 × 4.9–6.6 μ.

Hab. Ad *Thuja orientalem* L., ut folia ramulique moriantur efficiens. Baton Rouge, Louisiana, Amer. bor.

CONTROL

In the summer of 1943, a planting of arborvitae containing 107 diseased plants (59 Baker and 48 Berckman's Golden) in a nursery in Baton Rouge was divided into 4 sections and treated as follows:

Section I. 26 plants. Check, not treated.

Section II. 28 plants. Sprayed with 4–4–50 Bordeaux.

Section III. 27 plants. Sprayed with Cuprocide (89 per cent Cu) at the rate of 3 lb. in 100 gallons of water.

Section IV. 26 plants. Sprayed with Tennessee Copper Co. "Tribasic" copper sulphate (53 per cent Cu) at the rate of 6 lb. in 100 gallons of water.

Four spray applications were made, on June 15, July 19, August 4, and September 3.

Since the severity of the disease varied, each plant was examined carefully at the start of the experiment, and marked light, medium, or severe. This was a rough classification based on gross observation, but proved helpful in evaluating the results of the experiment.

All 3 sprays gave practically complete control. The progress of the disease on the sprayed plants was completely checked, and the new growth developing after spraying remained healthy. There was no shedding of branchlets. In the nonsprayed check plants, on the other hand, the disease continued to spread. The little new growth that developed became diseased. By the end of October, the ground under the nonsprayed plants was littered

with fallen diseased branchlets. Two plants (both Berckman's Golden) were completely killed. Many of the plants whose disease condition was marked light at the start of the experiment were in the medium or severe classes by October.

The contrast between the sprayed and nonsprayed plants was most striking in a section of 21 plants of the Baker variety. At the start of the test, all were classed as light in respect to disease. Eleven were sprayed with 4–4–50 Bordeaux and the other 10 were left as checks. By the end of October, the check plants were classed as severe; there was considerable spread of the blight upward and much shedding of necrotic branchlets. The sprayed plants, on the other hand, were practically normal; the progress of the disease was completely stopped, and the new growth remained healthy.

Although more work must be done to determine the optimum time to spray, the minimum number of applications for effective control, etc., the results of this one experiment were clear-cut enough to show that probably the disease can be controlled by spraying.

The Bordeaux spray left a rather heavy deposit on the plants which might be considered objectionable until toned down by weathering, although from a distance the Bordeaux-sprayed plants appeared deeper green and healthier. The Cuprocide imparted a light green, slightly rusty hue to the plants, but this was not prominent enough to be objectionable. The Tribasic spray residue, being greenish-blue, was hardly visible even at close inspection.

After this paper was prepared for publication, J. A. Pinckard brought to the writer's attention a publication (5) in which it is reported that, although the cause of the disease was not known, effective control was obtained in Mississippi by three separate agencies working independently. Copper sprays were used by all three. T. G. Owen and Son, nurserymen, Columbus, Mississippi, were the first to control the disease by spraying with Cuprocide. Paul V. Siggers, Division of Forest Pathology, U. S. Department of Agriculture, obtained control by using 4–4–50 Bordeaux at approximately monthly intervals. M. L. Grimes and J. A. Pinckard used 3 different copper sprays, 4–4–50 Bordeaux, Cuprocide, and basic copper sulphate, the last two at 3 lb. per 100 gallons of water. All 3 sprays gave satisfactory control.

SUMMARY

A destructive disease of oriental arborvitae, known locally as "blight" or "fire," is characterized by necrosis and shedding of foliage and branchlets, and by the formation of bark cankers on small twigs.

The cause of the disease, demonstrated by pure culture inoculations, is a *Cercospora*, apparently an undescribed species. This is described under the name of **C. thujina,** n. sp.

Perithecia of *Mycosphaerella sp.* often were found on diseased arborvitae leaves and branchlets, either alone or in association with the *Cercospora*, and it was thought at first that this might be the ascigerous stage of the latter.

Repeated inoculations with several ascospore isolates of the *Mycosphaerella*, however, failed to cause infection, and it must be assumed, therefore, that this fungus bears no relation either to the *Cercospora* or to the disease.

A limited spraying test using 4–4–50 Bordeaux, cuprous oxide (Cuprocide, 89 per cent Cu) at the rate of 3 lb. in 100 gallons of water, and basic copper sulphate (Tennessee Copper Co. "Tri-basic," 53 per cent Cu) at the rate of 6 lb. in 100 gallons of water, checked completely the progress of the disease on diseased plants. These results indicate that the disease is amenable to control by copper.

DEPARTMENT OF PLANT PATHOLOGY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

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EFFECT OF SEED TREATMENT ON PROTECTION, RATE OF EMERGENCE, AND GROWTH OF GARDEN PEAS

L. D. Leach[1] and Paul G. Smith[2]

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The beneficial effects of certain mercury and copper compounds in reducing seed and seedling rots of peas have been known and used commercially for a number of years. Several authors have contributed information on the factors that influence pea seed decay and on the relative effectiveness of different fungicides. This information was summarized by Walker *et al.* (23) in 1940.

Recently several nonmetallic fungicides have been introduced and found to be highly beneficial as pea seed protectants. Additional information on the evaluation of both metallic and nonmetallic fungicides has been published by Sharvelle and Shema (19), McNew (12, 15, 16), Felix (6), Sharvelle *et al.* (20), and by Bayliss *et al.* (4). The cooperative tests conducted in 1940, 1941, 1942, and 1943, by the Committee for Coordination in Cereal and Vegetable Seed Treatment Research of the American Phytopathological Society have added valuable information on the use of seed treatments on several varieties of peas and in different parts of North America.

The soil organisms responsible for seed decay and seedling rot have been studied by several investigators (3, 7, 9, 10, 17, 18). Most authors concluded that species of *Pythium* were the principal causes of the disease. Some found that species of *Rhizoctonia*, *Phytophthora*, *Fusarium*, and *Botrytis* caused high mortality of pea seeds and seedlings. Most tests have been in naturally infested soils and information on the relative protective value of fungicides on pea seed against specific organisms is limited. In one of the few trials with specific organisms, McNew (13) tested red and yellow cuprous oxide, Semesan, and Spergon on peas in soil infested with *Pythium ultimum* and found them equally effective as protectants.

Injurious effects upon peas have been reported from seed treatment with copper, mercury, and zinc compounds. Horsfall *et al.* (8) reported that red copper oxide occasionally caused slight stunting, especially in soil deficient in organic matter, while Kadow and Anderson (12) observed some injury on Perfection and Wisconsin Early Sweet varieties. Cook (5) found that zinc oxide tended to stunt the pea plants. From observations and yield data on field plantings McNew (12) concluded that cuprous oxide may cause slight injury to peas under certain conditions. In the same trials he found that New Improved Ceresan injured Surprise, Green Admiral, and Wisconsin Early Sweet varieties and concluded that this fungicide was too injurious for use on peas.

[1] Associate Professor of Plant Pathology and Associate Plant Pathologist in the Experiment Station, University of California.

[2] Instructor in Truck Crops and Junior Olericulturist in the Experiment Station, University of California.

Increased yields from treated pea seeds have led to discussions as to whether the increases could be attributed entirely to disease control or whether they were produced in part by chemical stimulation. Horsfall (7) found that red copper oxide accelerated emergence of pea seeds from cold wet soil infested with *Pythium ultimum*. He also observed other evidences of stimulation on vegetable crops but concluded that some of the differences might be due to density of stand or freedom from disease, although the possibility of response to copper as a nutritional element was not excluded. Concerning this point Kadow and Anderson (10) stated that "the benefit from treated pea seed is not limited to damping off control but extends to the general welfare of the entire plant." The same authors report that treatment of pea seed may materially reduce stem and root rots, and also refer (1) to the importance to seedling vigor of protecting cotyledons against decay. It appears, therefore, that the additional benefits from seed treatment were due, chiefly, to disease control rather than chemical stimulation. After testing Spergon on canning peas in Minnesota, Sharvelle and Shema (19) reported that in addition to its protective value it appeared to stimulate growth, and in a later paper Sharvelle *et al.* (20) claimed that treating seed with Spergon increased the rate of emergence and produced greater vine length and more vigorous root development. It is significant that their comparisons were between Spergon-treated and nontreated seeds in field plots and, except in a few trials, not between different seed treatments.

The most direct evidence of chemical stimulation by Spergon was supplied by McNew (13, 15), who found that disease-free pea seed planted in steamed soil produced 5 to 20 per cent more dry matter when treated with Spergon than when nontreated or treated with other fungicides, and in another report (14) stated that Spergon "as a growth stimulant . . . should pay dividends in practically every field, irrespective of growth conditions." In a repetition of his earlier trials, however, McNew[3] secured no significant differences in the seedling weights from various seed treatments in steamed soil.

The present investigations were to determine (1) the relative protective value of several metallic and nonmetallic compounds under controlled conditions with known organisms and under field conditions, and to determine (2) whether under local conditions any of these materials produced injurious or beneficial growth effects aside from protection from decay organisms.

MATERIALS AND METHODS

The experiments were in three series: (1) greenhouse trials with sterilized soil inoculated with cultures of *Pythium ultimum* or *Rhizoctonia solani*, (2) field plantings at various seasons of the year, and (3) greenhouse trials in sterilized soil.

For treatment, the required amount of seed was weighed out and placed in 1-quart fruit jars provided with a wooden baffle to insure adequate mixing

[3] Studies on Vegetable Seed Treatments in 1943. Plant Disease Reporter, Supplement 145: 11. 1944.

of the seed. After adding the desired quantity of chemical, these jars were rotated on a motor-driven device until the seeds were thoroughly coated with dust. Since these jars had been used previously for the same seed treatment, they were thoroughly coated on the inside with the particular chemical used and very little loss or addition of dust was to be expected.

The soil used in the tests with specific organisms was first pasteurized by passing it through the electrically heated device described by Tavernetti (21). In repeated trials this apparatus has eliminated the common damping-off fungi from soils when proper temperature and soil moisture are maintained. After pasteurization the soils were infested with either *Pythium* or *Rhizoctonia* by adding agar cultures of the fungus. A Waring Blendor was used to suspend the agar cultures in sterile water, as suggested by Andrus (2). The fungus suspension was mixed with the pasteurized soil and incubated for 5 days at about 20° C. before the seeds were planted.

PROTECTION AGAINST SPECIFIC ORGANISMS

In previously sterilized soil thoroughly infested with *Pythium ultimum*, greenhouse trials were run in the winter of 1942 and 1943. The experiments consisted respectively of 5 and 6 randomized replicates with 15 seeds for each plot in each replicate. The soil was well moistened before planting, and prevented from drying by a light watering as needed. Daily counts were made during the period of emergence. About 4 weeks after planting, the plants were dug, washed, and inspected. In order to express the severity of infection by a single figure, the following system of rating was used: no infection, 0; one cotyledon decayed, or slight damage, 25; both cotyledons decayed or moderate damage, 50; post-emergence damping-off, 75; and pre-emergence damping-off, 100. The disease index for each plot was obtained by multiplying each class value by the number of plants in that class, adding the products, and dividing the total by the number of seeds planted in each plot. A slight error is introduced by non-viable seeds being classed as pre-emergence damping-off but with high germinating seed this error would be small and about equal in all treatments.

In 1942 all five treatments gave a highly significant increase in emergence and freedom from infection as compared with the nontreated seed (Table 1). Semesan and Yellow Cuprocide provided the best protection with Spergon, New Improved Ceresan, and Arasan apparently somewhat less effective. The few nontreated seedlings that emerged had cotyledon rot or epicotyl infection, and small stunted plants resulted. The average weight of green seedlings (Table 1) has an inverse relation to the disease index. When the cotyledons were removed, seedling weights were more nearly equal for the various treatments.

In 1943 all treatments were again much better than the control but between treatments most of the differences in emergence were nonsignificant and with respect to the disease index they are of doubtful significance. As in 1942 seedling weight was highest after treatment with Semesan and

Yellow Cuprocide but this advantage was due largely to the weight of cotyledons (Table 1). This is an indication of the high protective value of these two chemicals against Pythium infection but trials in sterile soil, reported later in this paper, show, in addition, evidence of direct chemical effects upon the cotyledons.

The two years' experiments in soil infested with *Pythium ultimum* indicate that against this particular organism, Yellow Cuprocide and Semesan

TABLE 1.—*Relation of pea seed treatments, on the variety Laxton's Progress, to protection and growth in soil infested with Pythium ultimum*

Planted in greenhouse Dec. 16, 1942

Treatment	Dosage	Emergence	Disease index	Average weight per seedling		
				Green		Dry[a]
				With cotyledons	Cotyledons removed	Cotyledons removed
	Per cent	*Per cent*		*grams*	*grams*	*grams*
Nontreated		5.6	97.8	0.68[b]	0.50[b]	0.035[b]
Spergon	0.187	72.2	36.6	1.58	1.10	0.117
New Improved Ceresan	0.094	78.9	38.9	1.54	1.10	0.118
Yellow Cuprocide	0.187	92.2	12.0	1.73	1.18	0.118
Semesan	0.312	92.2	11.4	1.76	1.28	0.124
Arasan	0.187	51.1	63.6	1.35	1.01	0.115
Significant difference	Odds 19:1	12.1	11.8	0.18	0.17	n.s.
	Odds 99:1	16.3	15.9	0.24	n.s.[c]	n.s.
Planted in greenhouse Nov. 26, 1943						
Nontreated		17.3	88.0	0.84[b]	0.60[b]	0.08[b]
Spergon	0.187	66.7	44.7	1.83	1.64	0.162
New Improved Ceresan	0.094	73.4	39.3	1.95	1.69	0.166
Yellow Cuprocide	0.156	78.7	28.3	2.16	1.74	0.157
Semesan	0.25	70.7	35.0	2.14	1.77	0.169
Arasan	0.187	74.7	39.7	1.56	1.36	0.138
Significant difference	Odds 19:1	13.7	13.6	0.26	0.20	0.018
	Odds 99:1	18.7	18.5	0.36	0.30	0.024

[a] Dried to constant weight at 70° C.
[b] Not included in statistical analysis because of small number of surviving plants.
[c] Differences not significant.

gave the most effective protection. Spergon, New Improved Ceresan, and Arasan were only a little less effective.

Recently ter Horst and Felix (22) reported the high fungicidal potency of the compound "604" (2,3,dichloro-1,4-naphthoquinone) as a protectant for peas. During the present investigations, in a single greenhouse test in soil naturally infested with *Pythium ultimum*, the percentages of emergence were as follows: Nontreated, 11; "604" at a dosage of 0.2 per cent, 79; "604" at 0.1 per cent, 67; "604" at 0.05 per cent, 54; Spergon at 0.2 per cent, 47; and Semesan at 0.25 per cent, 82.

These results indicate that the new compound "604" is highly effective as a protectant for peas in soil infested with *Pythium*.

Similar trials were made with soil infested with an isolate of *Rhizoctonia solani* pathogenic on bean and beet seedlings but not tested on peas. In two experiments there was little pre-emergence damping-off, perhaps because conditions were unfavorable for infection. As a result there were no significant differences in emergence between nontreated and treated lots. Post-

TABLE 2.—*Relative protection, rate of emergence, and dry seed yield resulting from seed treatments in field plantings*

Treatment	Dosage	Emergence	Mean emergence period	Average yield of dry seed	
				Per plot	Per plant
	Per cent	*Per cent*	*Days*	*Grams*	*Grams*
Variety Laxton's Progress. Planted Jan. 14, 1943					
Nontreated	.....	58.3	26.6	532.7	9.14
Spergon	0.187	85.6	25.4	651.0	7.58
New Improved Ceresan	0.094	82.8	25.9	593.0	7.18
Yellow Cuprocide	0.187	83.0	32.0	565.5	6.82
Semesan	0.312	88.3	26.4	634.5	7.18
Significant difference	Odds 19:1	5.6	2.4	48.4	0.75
	Odds 99:1	7.6	3.3	66.0	1.02
Variety Wisconsin Early Sweet. Planted Jan. 14, 1943					
Nontreated	.....	18.5	24.8	185.2	10.01
Spergon	0.187	55.1	28.0	451.2	8.14
New Improved Ceresan	0.094	41.8	30.4	339.3	8.19
Yellow Cuprocide	0.187	43.0	30.8	274.5	6.37
Semesan	0.312	58.0	28.4	441.7	7.62
Significant difference	Odds 19:1	5.0	1.4	61.2	1.31
	Odds 99:1	6.7	1.9	83.4	1.79
Variety Laxton's Progress. Planted Aug. 16, 1943					
Nontreated	.....	29.3	8.95	.....	.....
Spergon	0.187	64.8	9.48	.....	.....
New Improved Ceresan	0.094	56.5	8.88	.....	.....
Yellow Cuprocide	0.187	70.7	9.12	.....	.....
Semesan	0.312	81.0	9.50	.....	.....
Significant difference	Odds 19:1	7.9	n.s.[a]	.....	.....
	Odds 99:1	10.8	n.s.	.....	.....

[a] Difference not significant.

emergence damping-off, however, was severe in all cases. Inspection of the seedlings showed a high degree of cotyledon rot and stem rot, regardless of the treatment. In these experiments, none of the materials was effective in controlling cotyledon rot and post-emergence rot caused by *Rhizoctonia*. Additional trials would be necessary to determine to what extent these results were influenced by the strain of the fungus or by environmental conditions.

FIELD EXPERIMENTS

The field experiments involved four different planting dates and five varieties of peas. The January and August plantings (Table 2) represent

the approximate weather conditions encountered during the two commercial pea planting seasons in the interior valleys of California. The February and March plantings (Table 3), conducted as part of the cooperative tests arranged by the Committee on Coordination of Cereal and Vegetable Seed Treatment Research of the American Phytopathological Society, represent periods too late for the spring pea crop.

In addition to the usual data on emergence and yield the writers have found the rate of emergence to be an excellent indicator of the effect of seed treatment. The most satisfactory measure of the emergence rate is the coefficient of velocity of emergence proposed by Kotowski (11). The first requisite is that the emergence stand be counted frequently during the germination period, preferably at daily intervals. Each daily increase in emergence is multiplied by the number of days since planting; the sum of these products is divided into the total emergence at the end of the trial and the quotient is then multiplied by 100 to give the coefficient of velocity of emergence.

The rate of emergence can be measured also by the mean emergence period, calculated in the same way as the coefficient of velocity except that the sum of the products is divided by the total emergence at the end of the trial. Also, the mean emergence period can be determined by dividing the coefficient of velocity into 100 since the former term is the reciprocal of the latter times 100.

The mean emergence period represents the weighted mean time required for the emergence of all seedlings for the treatment. Such a figure is obviously influenced by the seed, the depth of planting, moisture, temperature, and seed treatment. Within any one experiment all but the last factor can be assumed to be reasonably uniform. The writers have found the mean emergence period to be a more useful unit than the coefficient of velocity of emergence because differences between treatments in the same experiment represent the actual number of days by which the mean germination of one lot was delayed as compared to another lot.

The January planting in 1943 consisted of two varieties: Laxton's Progress and Wisconsin Early Sweet each tested with nontreated seed and four seed treatments. Each treatment was replicated six times and each plot consisted of a single row planted with 100 seeds. Between planting and emergence 7.8 inches of rain fell. The air temperature varied from a minimum of 21° F. to a maximum of 62° F. with a mean of 47° F., while the soil temperature at the depth of planting varied from 32° F. to 57° F. with a mean of 45.5° F. The first seedlings emerged 21 days after planting. In emergence of Laxton's Progress, there was no significant difference between the treatments (Table 2) but all were very much better than the nontreated control. Isolations from nontreated pea seeds removed from the soil before emergence indicate that *Pythium ultimum* was the pathogen chiefly responsible for pre-emergence damping-off in the early spring plantings. The data on mean emergence period (Table 2, column 4) show clearly that Yellow

Cuprocide delayed emergence by at least five days as compared with non-treated seed or with other treatments. The yields suggest that Spergon and Semesan combined excellent protection with freedom from injurious effects while the high yield per plant and low yield per plot in the control is undoubtedly due to the low density of stand. Yellow Cuprocide, in addition to delaying emergence, reduced the yield per plant and yield per plot.

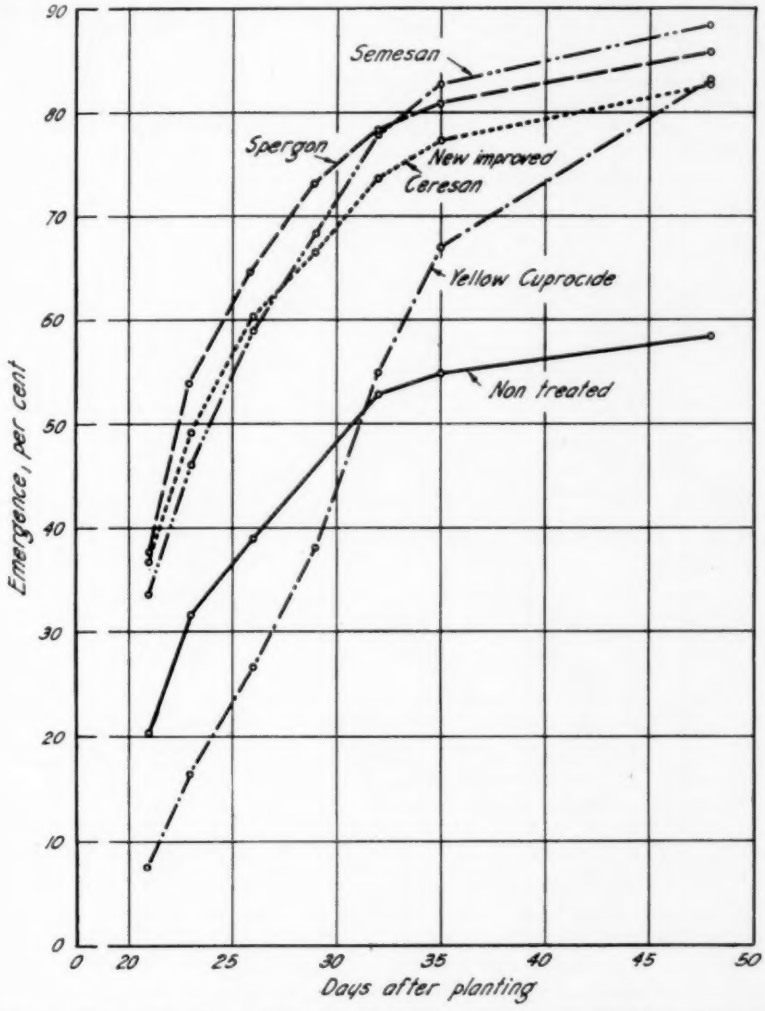

FIG. 1. Emergence curves for Laxton's Progress peas treated with seed protectants and planted at a low temperature. All treatments gave adequate protection but Yellow Cuprocide delayed emergence.

With Wisconsin Early Sweet the highest emergence was produced by Spergon and Semesan although all treatments produced much better stands than the control. Both Yellow Cuprocide and New Improved Ceresan appeared to delay emergence as compared with the other treatment and the

lower emergence from these treatments may therefore be due either to lower protective value or to injurious effects.

The rapid emergence of nontreated seed suggests that all treatments delayed germination but reference to the emergence curves (Figures 2 and 3) suggests another explanation. Apparently pre-emergence damping-off

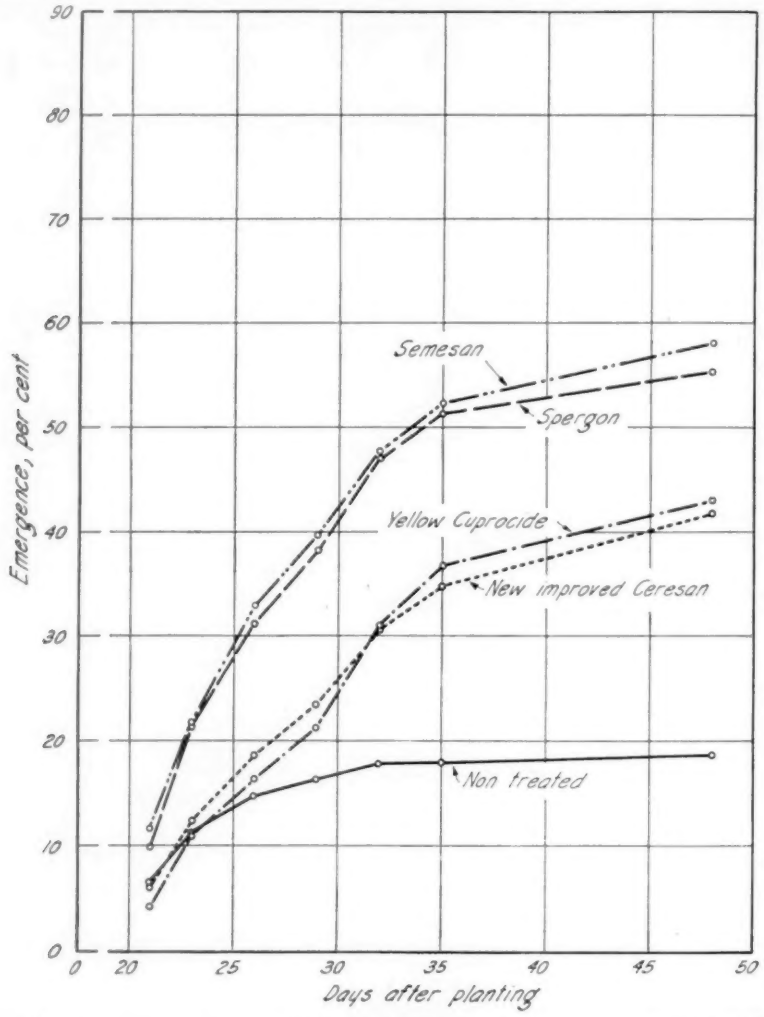

FIG. 2. Emergence curves for Wisconsin Early Sweet garden peas treated with seed protectants and planted at low temperature. The emergence rates were influenced by both protective effects and chemical injury.

was so severe that only the early germinating plants emerged, while most of the late germinating seed rotted below the surface of the soil. By contrast treatment of the seed provided protection for the late germinating seed. It is obvious, therefore, that comparisons between rates of emergence with different treatments should be limited to plantings in noninfested soil or to comparisons of treatments that provide nearly equal protection.

Because of low density of stand the nontreated control again had the highest yield per plant but the lowest yield per plot. The low per plant yield from seed treated with Yellow Cuprocide again suggests chemical injury while the yields from the other three treatments were in proportion to the density of stand.

The August planting of Laxton's Progress was made in the period during which the seed for the fall crop of peas is planted in Central California. In contrast to the low temperature that prevails during the spring plantings,

TABLE 3.—*Effect of seed treatment on stand and yield of garden peas. These data were obtained as part of the cooperative trials for the Committee for Coordination in Cereal and Vegetable Seed Treatment Research*

Varieties Surprise and Alderman. Planted Mar. 18, 1942

Treatment	Dosage	Surprise	Alderman		
		Emergence	Emergence	Green weight[c]	
				Vines and pods	Pods
	Per cent	*Per cent*	*Per cent*	*Grams*	*Grams*
Nontreated	.....	42.2	34.8	672	222
Red Cuprocide	0.279	74.8	83.0	1528	534
Yellow Cuprocide	0.168	79.4	85.4	1628	554
Semesan	0.279	84.2	87.4	1614	496
Spergon	0.223	83.0	83.4	1554	531
Significant difference	Odds 19: 1	7.8	7.8	310	93
	Odds 99: 1	10.8	10.8	427	128
Variety Thomas Laxton. Planted Feb. 16, 1943					
Nontreated	.....	.....	76.2	1930	1120
Arasan	.....[a]	.....	85.1	2400	1374
Fermate	.....[a]	.....	83.3	2347	1344
Spergon	.....[a]	.....	86.6	2400	1355
Significant difference	Odds 19: 1	.....	5.26[b]	310[b]	n.s.
	Odds 99: 1	.....	7.06	420	n.s.

[a] Emergence and yields based on averages for 3 dosages. Arasan, Fermate, and Spergon were each used at the following dosages: 0.084 per cent, 0.168 per cent, and 0.335 per cent. Differences between dosages and interaction of dosage and treatment were nonsignificant.

[b] Differences required for significance between treatment means and nontreated; differences between treatment means nonsignificant.

[c] Yields for this experiment are very low because of the late planting date.

soil temperatures are high and high soil moisture is provided by irrigation either before or after planting. It is also very probable that different seed decay and damping-off organisms are active at this time of the year. In this trial the soil temperature at the depth of planting varied from a minimum of 57° F. to a maximum of 94° F. with a mean of 73.4° F. The first seedlings emerged on the sixth day. Only stand and rate of emergence records were taken. The best stand was secured from Semesan-treated seed followed by Yellow Cuprocide, Spergon, and New Improved Ceresan in that order. Under the high temperature prevailing, the germination of all lots was comparatively rapid and none of the treatments appeared to depress the rate of emergence.

Seed treatments on three additional varieties are compared in table 3. On all three varieties, Surprise, Alderman, and Thomas Laxton, each seed treatment produced a significant increase in stand over the control but there were no significant differences in stand between treatments except that Red Cuprocide appeared to be less effective on Surprise than Semesan or Spergon. On both Alderman and Thomas Laxton all treatments increased the yield as compared with nontreated seed but there were no significant differences between treatments.

The fact that the three different chemicals used in the 1943 planting produced nearly equal increases in yield indicates that the result was due to protective values and not to the specific stimulating effects of any of the chemicals.

TRIALS IN PASTEURIZED SOIL IN THE ABSENCE OF DISEASE

To measure the effect of seed treatments on the rate of emergence and growth of pea seedlings in the absence of disease two greenhouse plantings were made in soil that had been pasteurized to eliminate pathogenic fungi.

In the first trial, seed treated with Spergon and New Improved Ceresan

TABLE 4.—*Rate of emergence and plant growth in sterile soil of treated pea seed, Variety Laxton's Progress, grown in flats in the greenhouse*

Planted Jan. 15, 1943

Treatment	Dosage	Emergence	Mean emergence period	Average weight per seedling			
				Green plants			Dried plants[a]
				With cotyledons	Cotyledons removed	Cotyledons only	Cotyledons removed
	Per cent	*Per cent*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*
Nontreated	.....	97.4	6.90	2.49	2.04	0.45	0.156
Spergon	0.187	95.4	7.00	2.54	2.04	0.50	0.162
New Improved Ceresan	0.094	97.4	7.12	2.52	2.05	0.47	0.159
Significant difference	Odds 19:1	n.s.[b]	n.s.	n.s.	n.s.	n.s.	n.s.
	Odds 99:1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Planted Dec. 3, 1943							
Nontreated	.....	93.4	8.71	2.33	2.11	0.227	0.159
Spergon	0.187	90.0	8.78	2.44	2.16	0.276	0.158
New Improved Ceresan	0.094	86.7	8.56	2.36	2.12	0.246	0.158
Yellow Cuprocide	0.156	90.7	9.39	2.17	1.85	0.328	0.143
Semesan	0.25	97.4	8.85	2.36	2.04	0.320	0.145
Arasan	0.187	92.7	8.59	2.35	2.11	0.235	0.159
Significant difference	Odds 19:1	6.4	0.43	0.14	0.14	0.034	0.011
	Odds 99:1	.....	0.58	0.19	0.19	0.045	0.015

[a] Dried to constant weight at 70° C.

[b] Difference not significant.

FIG. 3. Effect of seed protectants upon cotyledons of Laxton's Progress garden peas in pasteurized soil. Upper left—nontreated; Upper right—Spergon; Lower left—Semesan; Lower right—Yellow Cuprocide. The dark necrotic areas on the cotyledons in the lower row correspond to the ridges of the wrinkled peas. Spergon did not injure cotyledons, Semesan caused slight necrosis and reduced the rate of cotyledon absorption, while Yellow Cuprocide caused pronounced necrosis, delayed absorption, and reduced seedling growth.

were compared with nontreated seeds in 5 replications each containing 30 seeds. Conditions favored rapid germination and the results in table 4 show that neither of the treatments affected the emergence percentage, the emergence rate, or the weight of seedlings after 3 weeks of growth.

The second trial in pasteurized soil consisted of five seed treatments and a control each replicated 10 times with 15 seeds per row. The seeds were disinfected with mercuric chloride before treatment. Emergence was at about the same rate with all treatments except Yellow Cuprocide with which there was a significantly longer emergence period. It was also the only treatment for which there was a significantly lower seedling weight either with or without cotyledons. The dry weights of seedlings without cotyledons show that seeds treated with Yellow Cuprocide or Semesan produced smaller plants than seeds with other treatments or the control. At least part of this difference, however, is explained by the greater cotyledon weights for the same two treatments (Table 4, column 7).

The cotyledons of plants from the control or those treated with Spergon, Arasan, or New Improved Ceresan were shriveled and much reduced in size within 3 to 4 weeks after planting in the greenhouse. The cotyledons from plants in the Yellow Cuprocide and Semesan treatments were at the same time not shriveled and only slightly absorbed. Particularly upon the Yellow Cuprocide-treated seeds a very pronounced mottled reddish-brown pattern was evident (Fig. 3), this pattern apparently coinciding with the ridges on the wrinkled seed. The sunken areas of the dry seed are less heavily coated with the dust and remain green after the seed swells during germination. The darkened pattern, as shown by microscopic examination, is composed of necrotic cells on the surface of the cotyledons and results from the heavy dosage of the dusts on these areas. With Semesan-treated seed the cotyledon necrosis is mild and apparently has not reduced the total seedling weight or adversely affected subsequent growth. With Yellow Cuprocide, however, the seedling weights were significantly less than with other treatments and as shown in table 2 the yield of seed per plant was reduced. This reduction may have been due to retarded emergence or to the reduced rate of cotyledon absorption or to a combination of both factors.

DISCUSSION

Pea seed were treated with a number of commercial compounds to determine the effect of these materials under some of the planting conditions in California, their effectiveness as protectants against *Pythium ultimum* and *Rhizoctonia solani*, and their effect on plant growth. In addition to the usual emergence percentage, other measures of the effect of the seed protectants were used in some experiments. A disease index combines the degree of injury to the living seedling, and the incidence of pre- and post-emergence damping-off. This figure was, in general, closely related to the stand counts. In some of the experiments the relative rates of emergence were measured by a mean emergence period.

Most trials with seed protectants have been in field soils without knowledge of the specific organisms involved. Because some fungicides are more or less specific in their toxicity relations it is important to measure their protective values in soils infested with different pathogens.

In three greenhouse tests in soil infested with *Pythium ultimum,* the highest protective values were given by Semesan and Yellow Cuprocide; but Spergon, New Improved Ceresan, and Arasan were only slightly less effective. In a single trial a new material, dichloro-naphthoquinone (604) produced excellent stands in heavily infested soil.

In field plantings during the early spring months *Pythium ultimum* appeared to be the chief cause of seed decay but the infestation was much lighter than in the greenhouse trials. In the field, Spergon appeared to be as effective as Semesan and in some trials had definite advantages over Yellow Cuprocide and New Improved Ceresan. Trials with Arasan and Fermate were too limited to justify definite conclusions.

Two greenhouse tests were conducted in soil artificially infested with *Rhizoctonia solani.* Due perhaps to environmental conditions or to the strain of the fungus, infection was limited to post-emergence rot of cotyledons and epicotyls. None of the seed treatments effectively controlled this phase of infection.

The rate of emergence as measured by the mean emergence period or by the coefficient of velocity of emergnce was adversely affected by certain seed treatments. Under warm temperatures, favorable for rapid germination, none of the treatments significantly altered the mean emergence period; but whenever the germination period was prolonged by low temperatures Yellow Cuprocide caused a significant delay in emergence and a reduction in subsequent growth.

In one field experiment planted in January, the emergence of two varieties, Laxton's Progress and Wisconsin Early Sweet, was delayed 5.4 days and 2.8 days respectively by Yellow Cuprocide. A prolonged injurious effect was reflected by a significant decrease in total yield, and yield per plant of dried seed at the end of the season. In two greenhouse experiments, one in sterile soil and one in *Rhizoctonia*-infested soil where temperatures were sufficiently cool, emergence was delayed; and seedling growth in the sterile soil was definitely reduced.

There was some indication of injury by New Improved Ceresan, although the evidence was not so clear as with Yellow Cuprocide. In a field planting of Wisconsin Early Sweet, treatment with New Improved Ceresan caused a definite delay in emergence. In two of three field tests, stands from seed treated with New Improved Ceresan were significantly less than for one or more other treatments. It is not clear, however, whether this was due to injury or to a lower degree of protection. No evidence of injury from the use of Spergon or Arasan was observed in any of the trials, and Semesan produced only slight cotyledon necrosis and some delay of cotyledon absorption without significantly affecting seedling growth or yield of mature plants.

Because other workers have reported evidence of stimulation from treating pea seeds with Spergon this point was tested both in greenhouse plantings in pasteurized soil and in field plantings. In pasteurized soils the results were influenced only by injurious or stimulatory effects since protective effects did not occur. In these experiments there was no evidence that any of the materials tested significantly increased the rate of emergence or the green or dry weight of seedlings.

In field trials both the rate of emergence and yield may be influenced by infection and by relative protective effects of the fungicides, as well as by injurious or stimulatory effects. It is evident from three field trials that none of the treatments hastened emergence as compared with nontreated seeds or with other noninjurious treatments. Yield data from three field plantings with different varieties show no significant differences between Semesan and Spergon. Yellow Cuprocide and New Improved Ceresan, however, resulted in significantly lower yields in two of the three tests. In a single test with a low incidence of infection there was no significant difference in the emergence rate or yield of peas grown from seed treated with Arasan, Fermate, or Spergon although each treatment improved both stand and yield of vine and pods as compared with the nontreated control.

The writers conclude, therefore, that under the conditions of these tests the benefits of seed treatment were due entirely to disease prevention. In the absence of infection none of the seed treatments tested produced more rapid germination or higher yields than did the nontreated control. In the presence of pathogenic organisms no treatment produced significantly higher yields than other treatments that were equally protective and equally noninjurious.

SUMMARY

These investigations comprise a study of the protective values of several commercial seed treatment preparations and their effect on rate of emergence, growth, and yield of garden peas.

A comparison of protective values shows that in soil artificially infested with *Pythium ultimum*, Semesan and Yellow Cuprocide appeared to provide better protection against infection than Arasan, New Improved Ceresan, or Spergon although the differences were not great and all five materials provided stands far superior to the nontreated control. In a single trial dichloro-naphthoquinone also appeared to be highly effective as a protectant against *Pythium ultimum*.

In similar trials in pasteurized soil artificially infested with a strain of *Rhizoctonia solani* that produces damping-off on sugar beets and beans, no seed decay or pre-emergence damping-off occurred upon peas. None of the seed treatments effectively controlled post-emergence epicotyl infection or cotyledon decay.

In field trials where infestations, principally *Pythium*, were light or of moderate intensity all of the chemicals provided adequate protection. Chiefly because of freedom from injurious effects, however, Spergon and

Semesan produced better results in some tests than Yellow Cuprocide or New Improved Ceresan.

Injurious effects, represented by reduced rates of emergence and seedling growth, were caused by Yellow Cuprocide when the seed was planted at low temperature. At moderate or high temperature when germination was rapid no injury by Yellow Cuprocide was apparent. New Improved Ceresan also appeared to delay emergence in one test but it is difficult to determine whether the reduced yield, as compared to those with Spergon or Semesan, resulted from mild injury or from a lower protective value.

Localized necrosis of cotyledons resulted from treatment of Laxton's Progress peas with Yellow Cuprocide and absorption from the cotyledons by the growing plant was delayed. Semesan also produced a mild form of localized cotyledon necrosis and delayed absorption but did not appear to reduce seedling growth or yield of mature plants.

No evidence of chemical stimulation as distinguished from fungicidal protection was observed in these tests.

CALIFORNIA AGRICULTURAL EXPERIMENT STATION,
DAVIS, CALIFORNIA.

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THE EFFECT OF MANURE AND OF COMMERCIAL FERTILIZER ON SUSCEPTIBILITY OF YOUNG APPLE TREES TO BLACK ROOT ROT

J. S. COOLEY[1]

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There is evidence[2] that certain conditions that presumably affect adversely the synthesis and translocation of organic materials and other life processes also affect susceptibility of apple trees to black root rot (*Xylaria mali* Fromme). It seemed desirable to get information on the effect of good nutrition versus poor nutrition on the susceptibility of apple trees to this disease; also to endeavor to determine to what extent the usual fertilizer elements influence susceptibility of the host.

EFFECT OF MANURE

In the spring of 1934 Wealthy and Early Ripe trees were set in two nursery plots at the U. S. Horticultural Station at Beltsville, Maryland, preparatory to manuring and inoculating with *Xylaria*. One plot received manure at the rate of about 14 tons per acre and the other received none. The manure was applied every year for 8 years except in 1938 and 1942. Cultivation continued until 1938 when it was discontinued on account of increased size of trees. Trees were inoculated first in 1936 and then each year through 1942, usually about the middle of July. The inoculations were made one year on one side and the next year on the opposite side of the main root. The method of recording size of lesion was the same as that used in a former publication.[3] In taking the record of percentage of infection and size of lesion the shallow lesions that involved only the cortex and usually persisted for a single year were recorded separately from the deep lesions that involved and killed the cambium. Since the percentage of cortical lesions usually corresponded to that of deep lesions, only the latter are presented in this discussion.

By the end of the first year or two the manured trees showed high vigor as evidenced by the large dark green leaves and by the twig elongation. However, there was no visual evidence of over-feeding. On the other hand the nonmanured trees, early in the experiment, began to show evidences of lack of vigor. By the end of the experiment these trees had less diameter and height than those in the manured plot. The general effect, however, in the nonmanured trees was that of low vigor rather than of a starved condition.

[1] Senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Bureau of Plant Industry Station, Beltsville, Maryland.

[2] Cooley, J. S. Some host-parasite relations of black root rot of apple trees. Jour. Agr. Res. [U.S.] 69: 449–458. 1944.

[3] Cooley, J. S. Factors affecting distribution and severity of black root rot of apple trees. Jour. Agr. Res. [U.S.] 65: 299–311. 1942.

The percentage of infection obtained in these 7 years of inoculations varied from year to year, it being usually between 60 and 80 per cent. The percentage of infections obtained in 1937 was lower on these as well as on other plots than that obtained in other years. The number of infections obtained in 1938 was also abnormally low. In these two years the percentage of infection was less on the manured than on the nonmanured plot. In 1937 the deep infections were only 9 per cent on the manured plot and 37 per cent on the nonmanured. In 1938 the manured plot showed 36 per cent infection and the nonmanured plot 57 per cent. However, in 1936, 1939, 1940, 1941, and 1942 the difference between the manured and nonmanured plots was negligible. The general average for the 7 years was 51 per cent infection on manured trees and 61 per cent on the nonmanured trees.

The yearly average diameter of lesion ranged from 18 to 40 mm. There was no striking difference in size of the lesion between the manured and nonmanured plots. The average size of lesion for all 7 years' inoculations on trees in the manured plot was 28 mm. and on trees in the nonmanured plot was 26 mm. In the main the infection percentage and the size of the lesions on the trees in this experiment were comparable to those on other experimental plots. In general, the data for seven years of consecutive inoculation show that manuring did not affect either favorably or unfavorably the incidence of the disease. No test has been made of the advance and persistence of the disease on bearing trees of high vigor compared with those of low vigor.

EFFECT OF COMMERCIAL FERTILIZER ON RESISTANCE OF APPLE TREES TO BLACK ROOT ROT

The trees used in this experiment were planted 12 to 20 inches apart in rows four feet apart. The experiment was begun after the trees had been planted three years and when they were beginning to show apparent nutrient deficiency. The seven fertilization combinations consisted of N–K–P, N–K, N–P, K–P, N, K, P, and check, each of which was replicated four times in a randomized arrangement. All fertilizers were applied in March or early in April. Each plot contained 12 linear feet of nursery row in which there were 9 to 12 trees, and the treatments were replicated 4 times, making a total of about 40 trees for each fertilizer treatment. The plots were arranged in four rows with buffer rows alternating with the 4 fertilized rows. The potassium, as muriate of potash, was applied in holes about 8 inches deep and 12 inches apart, 10 inches from each side of the tree row. Nitrogen, as Chilean nitrate of soda, and phosphorus, as 16 per cent acid phosphate, were applied on the surface. Assuming that the fertilized trees fed in the fertilized plot only and that the trees in the buffer rows did not appropriate fertilizers intended for experimental rows, the rate of application was 107 pounds of muriate of potash, 107 pounds of 16 per cent acid phosphate, and 324 pounds of nitrate of soda per acre. The only evidence of fertilizer injury was a yellowing of the foliage and slight defoliation on the plots receiving potash alone.

By the end of the experiment no outstanding differences could be noted in the different plots. Twig elongation and color and size of the leaves did not indicate outstanding differences in nutrient levels. The trees in the check plots did not make quite as much twig elongation as those in some of the nitrogen plots, but they were not in a starved condition.

The percentage of deep infection on the check plot for most years was comparable with the results of other inoculation experiments. In none of the fertilizer treatments did the percentage of infected trees differ greatly from that in the check plot.

During the 4 years the inoculations were made the infection percentage varied somewhat from year to year. In the 3 years 1939 to 1941 inclusive, it usually ranged around 55 to 80 for the various fertilizer treatments. In 1942, however, it was consistently low on all fertilized plots, ranging from about 20 to 30 with an average of 26, while the unfertilized check was 32. The average percentages of deep infections for the 4 years from 1939 to 1942, inclusive, were: check, 62; N–K–P, 62; N–P, 60; N, 61; K, 46; K–P, 67; P, 48.

The average percentages of infection on the plots receiving only potassium and on those receiving only phosphate were low. However, the plot receiving a combination of these two elements had as many lesions as the other fertilized plots or the check. The data indicate that there is no appreciable difference in the percentage of infection resulting from the fertilizer treatments used.

The average size of lesion in mm. for each fertilizer treatment was as follows: check, 26; N–K–P, 24; N–P, 28; N–K, 27; N, 27; K, 26; K–P, 25; P, 24. There was thus no appreciable difference in the size of lesion on the plots receiving the different fertilizer treatments.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

PULLULARIA PULLULANS STORAGE FRUIT SPOT OF TOMATO

CARLTON F. TAYLOR AND LELAND SHANOR

(Accepted for publication November 28, 1944)

Early in December, 1943, the senior author's attention was called to a spot of tomatoes that was developing on ripe fruit of the variety Break O'Day which had been in cold storage since the latter part of October at West Virginia University, Morgantown. Since the spot was one which was not familiar, isolations were made from fruits at Morgantown. Later a spotted fruit was left at the Beltsville, Maryland, laboratory of the Emergency Plant Disease Prevention Project for additional isolations. *Pullularia pullulans* (DeBy.) Berkhout (= *Dematium pullulans* DeBy.) was obtained independently in pure culture in both laboratories. Because this organism apparently has not been reported as a pathogen on tomato fruit, the spot caused by it is being illustrated and described briefly, together with a short account of experimental evidence demonstrating its pathogenicity.

The tomatoes on which the spots later developed were picked on October 6, following a light frost, and were placed on newspaper on a floor to ripen. As fruits ripened they were individually wrapped in oil-treated wrappers and placed in the cold storage room where the temperature was between 1° and 4.5° C. and where the humidity was high. Some of the wrappers had been used previously to wrap sweet potatoes, while others were being used for the first time. The fact that some wrappers had been used and others had not, did not influence the prevalence of the fruit spots. Spots were as abundant on fruit wrapped in unused wrappers as on fruit wrapped in wrappers that had been used before. By late November many of the fruits had characteristic spots and by the end of December practically all of the fruits were affected.

In the early stages of development spots caused by *Pullularia pullulans* appear superficially to be somewhat similar to early anthracnose lesions. At first, the mycelium forms a light colored mat under the epidermis, and the area immediately surrounding it appears water soaked (Fig. 1, a). A depression soon develops and eventually the mycelium in the center becomes deeply pigmented so that the center of the spot is black surrounded by a lighter region. This in turn is surrounded by a water soaked area (Fig. 1, b). Lesions are circular and seldom attain a diameter greater than 1.5 cm. Eventually the epidermis over infected areas cracks and the mycelium develops outwardly to form a relatively solid ridge of fungal tissue, at first light colored, but later becoming black and shining.

When the skin of fruit is peeled away from the flesh over lesions, the underlying fungal growth tends to remain attached to the epidermis in a somewhat hemispherical mass. Pure cultures were isolated easily from these fungal masses. Cultures of isolates grow readily on such standard culture media as potato-dextrose agar and corn-meal agar and produce conidia abun-

dantly at the surface of the culture media. Such colonies are smooth, shining, at first white to cream, later buff or tan, and in surface growth appear distinctly yeast-like. In older cultures, portions of the mycelium within the agar and scattered segments in the surface growth turn black. Such mycelium has rounded cells with much thickened and pigmented walls.

Conidia commonly sprout from any hyaline cell of the mycelium and frequently produce secondary conidia either while attached or upon becoming separated from the parent cell. Sprouting from secondary conidia and the conidia that continue to be formed in this manner often results in the formation of conidial chains. In order to demonstrate the pathogenicity of isolates and the ability of spores to penetrate the epidermis and develop

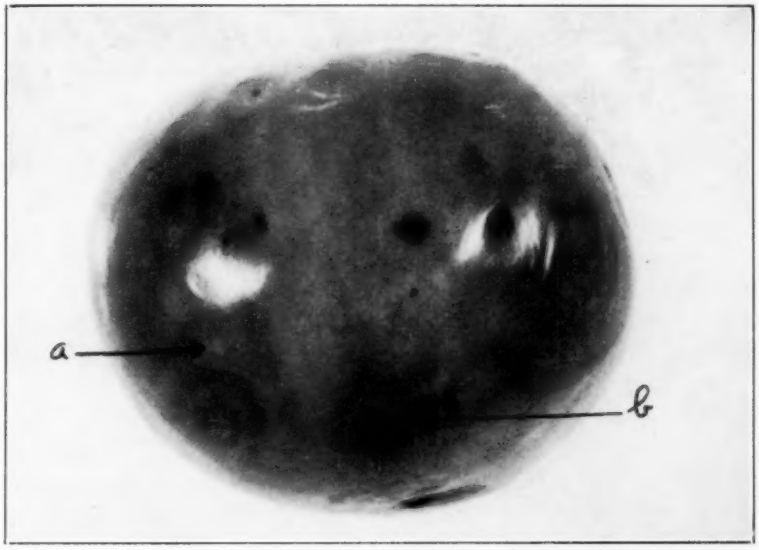

FIG. 1. Tomato fruit of variety Marglobe, showing fruit storage spot produced by *Pullularia pullulans*. a. Spot before pigmentation of the organism has developed. b. Later spot showing central area occupied by darkly pigmented mycelium surrounded by an area of lighter mycelium and an outer water-soaked region. ×about 1.7. (Photograph by W. J. Mead.)

under storage conditions, fruit of the variety Marglobe, after being washed in sterile distilled water, were dipped in a spore suspension, placed in moist chambers, and then allowed to remain in a refrigerator at 7.5° to 10° C. Fruit in varying degrees of maturity from green to ripe was inoculated. After about ten days in the refrigerator typical early lesions were evident on inoculated ripe and nearly ripe fruit. Three or four days later the typical darkly pigmented mycelium became evident in the center of many of these spots. The fruit illustrated in figure 1 was inoculated by being dipped in a spore suspension. Spots developed more slowly on green tomatoes than on ripe or nearly ripe fruit. The fungus developing under the epidermis of green fruit became pigmented almost from the time spots were recognizable. Pure cultures of *Pullularia pullulans* were reisolated from infected

fruit, thus demonstrating beyond a doubt that this fungus was responsible for the fruit spot and may be parasitic to tomatoes under the storage conditions noted.

The temperature of the room in which the spot was originally detected was below that at which tomato fruits are commonly stored and the temperature of the refrigerator used for storage of the inoculated fruit was also slightly below that generally recommended for commercial practice. Whether the parasitic tendencies of this organism for tomato are limited to low temperature environments alone has not been investigated.

BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING,
BELTSVILLE, MARYLAND.

DESIGN FOR CONSTANT-TEMPERATURE TANKS

W. A. Campbell and John T. Presley

(Accepted for publication November 20, 1944)

In the course of investigations on guayule diseases it was deemed desirable to study the relation of soil temperatures to infection by soil fungi. For this purpose constant-temperature tanks were built. These were patterned after those developed at the University of Wisconsin,[1] but their construction had to be greatly modified because of the unavailability of preferred materials. These modified constant-temperature tanks,[2] especially those cooled directly by mechanical refrigeration, proved so satisfactory that plans of their construction are herein presented.

The series consisted of six redwood tanks each divided into two compartments. Four of the tanks (8 compartments) were electrically heated and thermostatically controlled to provide temperatures above that of the water supply (approximately 70° F.). Four compartments in two tanks were cooled by individual refrigeration coils to provide temperatures below 70° F. The temperatures in both the electrically heated and the mechanically cooled tanks did not fluctuate more than 2 degrees from the desired temperature.

Each compartment contained 8 metal cans, 8 inches in diameter and 18 inches deep, in which plants were grown directly, or in 6-inch unglazed pots embedded in sand to facilitate aeration and drainage. Details of the construction of the redwood tanks and the arrangement of the cans are in figure 1. Blueprints of the tanks may be obtained from the Guayule Research Project.[3]

Construction of the Tanks.—The tanks were of 2-inch planed redwood planks splined with $\frac{1}{2} \times 1$-inch redwood strips and firmly bolted together by long iron rods. In order to replenish water lost by evaporation, as well as to provide a means of quickly changing the temperature within a tank, a $\frac{1}{2}$-inch water line with valve was connected to the bottom and an overflow pipe arranged at the top.

Electrically Heated Unit.—Each individual compartment in the four tanks designed for operation at temperatures above that of the water supply was electrically heated by 30 feet of lead-covered heating cable connected to a 110-volt line. The capacity of the heating cable was sufficient to furnish a temperature range from 70° to 110° F. The desired temperature within each compartment was maintained by an adjustable thermostat connected to each heating unit.

Mechanically Cooled Unit.—An ordinary refrigerating unit (Kelvinator

[1] Jones, L. R., J. Johnson and J. A. Dickson. Wisconsin studies upon the relation of soil temperature to plant disease. Wisc. Agr. Exp. Stat. Res. Bul. 71. 1926.

[2] The details of the construction of the tanks, as well as the arrangements for heating and cooling, were developed by M. G. Still, Chief Foreman, Construction and Maintenance, Forest Service, Emergency Rubber Project.

[3] The drawings of the constant temperature tanks were prepared by W. E. Deemer, Associate Mechanical Engineer, Forest Service, Emergency Rubber Project.

series SSB, powered by a ⅓-horsepower electric motor) furnished refrigeration for the 4 compartments in which temperatures below 70° F. were main-

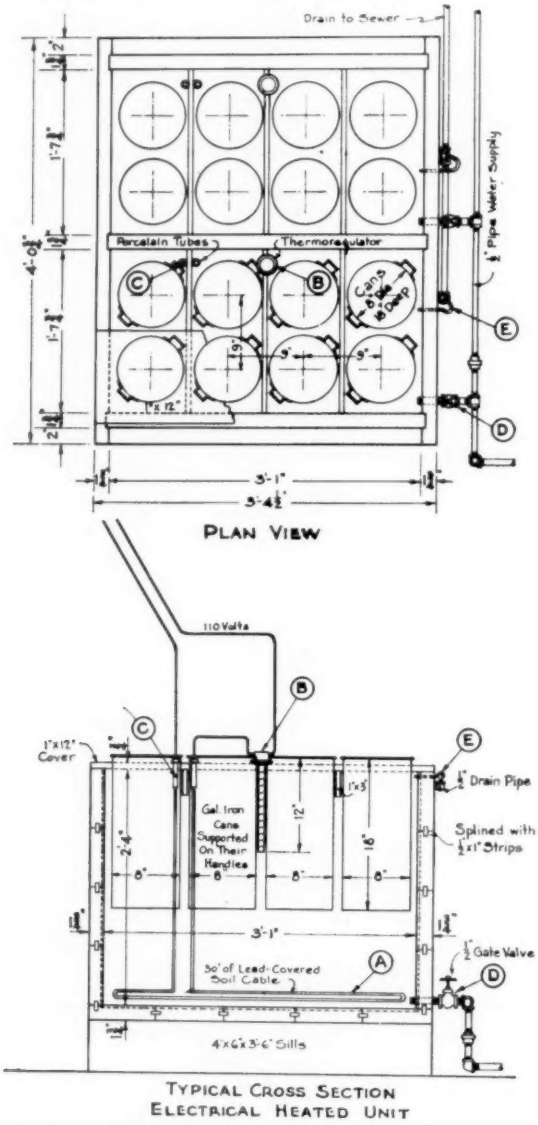

FIG. 1. Electrically heated constant-temperature tanks. Upper diagram: Plan view giving dimensions of redwood box and arrangement of galvanized cans. Lower diagram: Cross-sectional view illustrating arrangement of heating elements and thermostat. Installation of relays not illustrated.

A. 30 feet of lead-covered soil cable, 110-115 volt, 700 to 750 watts capacity. B. Thermostat (American Instrument Co., "Quickset" bimetal thermoregulator). C. Porcelain-tube insulators. D. Water intake at bottom. E. Overflow pipe of ¼-inch copper tubing emptying into ½-inch galvanized drain.

tained. This unit had sufficient capacity to maintain 2 compartments at 40° F. and 2 at higher temperatures, but would not maintain all 4 at 40° F.

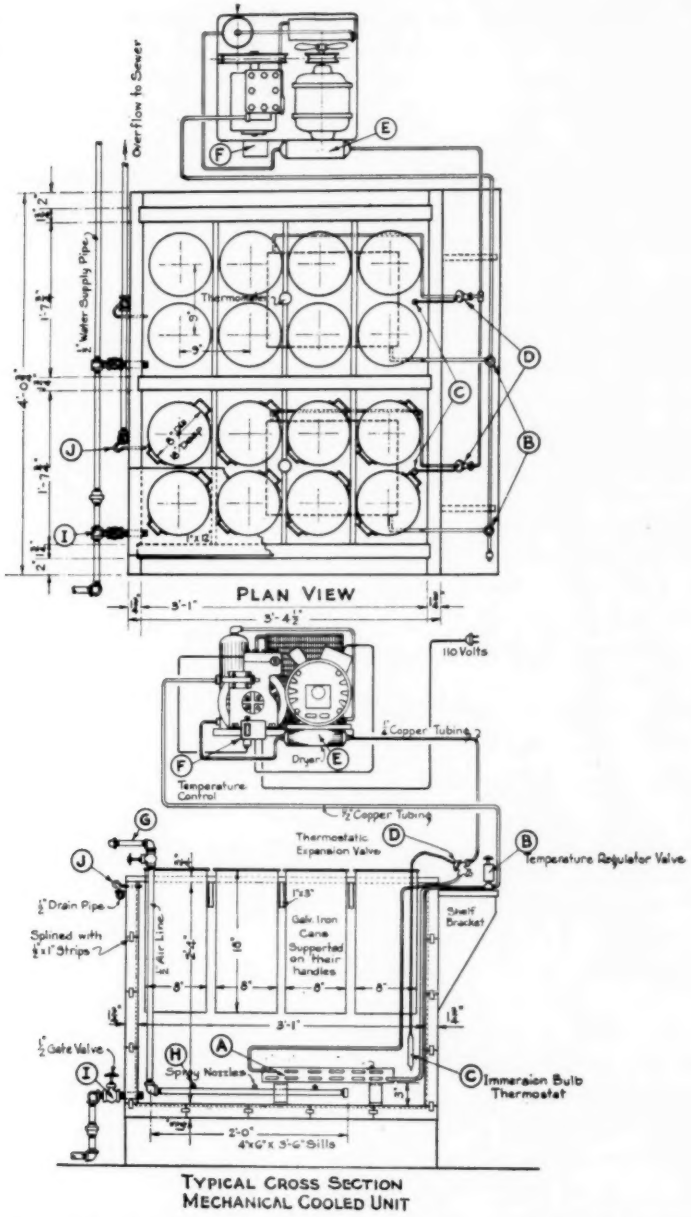

FIG. 2. Mechanically cooled constant-temperature tanks. Upper diagram: Plan view showing arrangement of tubing connecting two of the cooling coils with the refrigeration unit. The installation now in use has 4 compartments connected to the same refrigerating unit. Lower diagram: Cross-sectional view, showing details as to the arrangement of the installations in a single compartment.

A. Cooling coil. B. Temperature-regulator valve. C. Immersion-bulb thermostat. D. Thermostatic expansion valve. E. Dryer. F. Temperature control. G. Air line. H. Spray nozzles to provide agitation. I. Water intake at bottom. J. Overflow pipe (¾-inch copper tubing).

Installations at the refrigeration unit consisted of a dryer (E) and a temperature control unit (F) connected as shown in figure 2. The cooling coils (A), one to each compartment, were connected to the refrigerating unit. Each cooling coil was supplied with a temperature-regulator valve (B) and an immersion-bulb thermostat (C) operating in conjunction with a thermostatic expansion valve (D). Different temperatures could be maintained in each of the 4 compartments by adjusting each temperature-regulator valve. In actual practice temperatures from 40° to 65° F. were commonly maintained.

In order to maintain uniform temperatures within the tank, an air line (G) with 3 spray nozzles (H) was placed in the bottom of each compartment to provide agitation and circulation of the water.

SPECIAL GUAYULE RESEARCH PROJECT,
BUREAU PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING,
SALINAS, CALIFORNIA.

PHYTOPATHOLOGICAL NOTES

Poria microspora in House Timbers.—The urgent demands for wood during the war period have often resulted in the use of poorly seasoned lumber which, when shipped long distances in carload lots, sometimes arrives at retail yards with mats of fungus mycelium covering many of the timbers. This lumber is often sold to building contractors before much more air drying can take place, even in those yards where open piling is employed. The use of such partially dried and contaminated lumber in war-time housing construction, coupled with restricted ventilation within the walls of houses insulated by modern methods, creates moisture conditions that favor the growth of wood-decay fungi. Under such conditions a native but newly described fungus, *Poria microspora* Overholts,[1] has been found causing serious decay in a new house of modern construction at Syracuse, New York.

The fungus apparently was present in the lumber at the time of construction. Although the house was heated and ventilated immediately after plastering, the moisture conditions within the insulated walls were such as to permit decay. It is believed that moisture from the drying plaster raised the relative humidity of the air within the wall, and the uprights, sheathing, and inner surface of the siding absorbed sufficient moisture, either directly from the air or as a result of condensation, to permit active decay.

No sporophores were formed on the decaying wall timbers but when several boards of the decaying pine sheathing were placed in the decay cellars at the New York State College of Forestry fruit bodies were produced on the wood in approximately one year. A thick, light-colored mycelial mat appeared first and large drops of colored liquid were profusely formed upon its surface. Tubes formed soon afterwards. The determination of *Poria microspora* was made by the junior author and confirmed by Dr. L. O. Overholts of the Pennsylvania State College, and a further confirmation was made by means of cultural as well as morphological characters by Dr. Mildred K. Nobles, Junior Plant Pathologist, Division of Botany and Plant Pathology, Central Experimental Farm, Ottawa, Canada.

Poria microspora appears to form fruit bodies very rarely, as these have been reported from only three other widely scattered stations. It was first found on Sitka spruce in the Queen Charlotte Islands off the coast of British Columbia, and later on flooring at Sharon, Wisconsin, and on a southern pine beam in a boat at Mystic, Connecticut. Its existence in Eastern Canada at Calumet, P. Q., has been determined from cultures of spores caught in spore traps. Infected wood has been demonstrated over a still greater area, by cultures of Sitka spruce and Douglas fir imported into Great Britain from Canada, and from Douglas fir in Oregon and Toronto. So far it has been found in British Columbia only on standing trees.[2] This extensive but sporadic distribution probably indicates that the fungus is widespread but has

[1] Nobles, Mildred K. A contribution toward a clarification of the Trametes serialis complex. Canad. Jour. Res. (C) **21**: 211–234. 1943.

[2] Nobles, M. K. *Op. cit.*

been overlooked because of the rare appearance of the fruiting structure; perhaps also because the plant has been confused with *Trametes serialis* Fries.

Externally *Poria microspora* (Fig. 1) closely resembles the resupinate and more familiar *Trametes serialis*. The fruiting body, formed in the decay cellars, is dull white, about 3 mm. thick, and the texture when fresh was dry and soft-papery, quite different from the much tougher *T. serialis*. The

FIG. 1. A fruit body of *Poria microspora* produced on southern pine sheathing.

tubes reached a length of 8 mm. on a sloping surface, and the openings or pores averaged about 2–2.5 per mm. The subiculum hyphae bear abundant clamps. The spores are smooth, hyaline, oblong to oblong-ellipsoid, 5–6 × 2.5–3 μ. These microscopic characters serve to separate *P. microspora* from *T. serialis*, which lacks clamp connections on the subiculum hyphae and also has larger spores, 7–9 μ long.—RAY R. HIRT and JOSIAH L. LOWE, New York State College of Forestry, Syracuse, New York.

The Name of Ansatospora macrospora.—In the summary of his recent paper on a storage rot of celery, Newhall[1] states: "The fungus causing this

[1] Newhall, A. G. A serious storage rot of celery caused by the fungus *Ansatospora macrospora* n. gen. Phytopath. **34**: 92–105. 1944.

disease is thought to be the one previously described by Osterwalder as the cause of a pansy leaf spot, named *Cercospora macrospora* by him. Its identity with the fungus causing anthracnose of caraway, described and named *Cercospora cari* by Westerdijk and van Luijk, also is established. Its name is changed to *Ansatospora macrospora* (Ostw.) n. gen. chiefly on the basis of the long prominent appendage protruding from the basal cell of the conidium."

The purpose of this note is to point out that in 1880 Robert Hartig[2] described what is undoubtedly the same fungus as causing a destructive disease of maple seedlings. He called this organism *Cercospora acerina*. Later, Frank[3] made the statement that Hartig had determined the fungus wrongly and without further comments he named it *Sporidesmium acerina* (Hart.) Frank. It is presumed that Frank examined some very old material in which the conidia are quite dark and frequently swollen. Still later Arnaud[4] observed the fungus on maple seedlings in France but evidently only in the young, hyaline condition, for he changed the name to *Cercosporella acerina* (Hart.) Arn. Like Osterwalder,[5] Hartig failed to mention or recognize the conidial appendages as such, though these structures are clearly shown in the illustrations of both authors. That Hartig actually saw the appendages is clear from his drawings and from his comments. He states that if the slender tip of a conidium is broken before growth is completed, then a substitute tip grows out somewhere near the end. If this substitute tip occurs on the slender part of the conidium, it grows out at a right angle, and if it occurs lower down on the broken conidium then it grows out "bayonet like." In his own words: "Bricht vor Beendingung des Wachstums die zarte Spitze ab, dann wächst nahe dem Ende seitlich eine Ersatzspitze hervor, die dann, wenn sie an dem zarten Theile der Conidie sich bildet, rechtwinklig, Fig. 5a, wenn sie an einer tiefer unten abgebrochenen Conidie entsteht, bajonettartig hervortritt Fig. 5b." He evidently misinterpreted the appendages for outgrowths in response to injuries. Hartig gave no spore measurements other than those indicated by the stated magnifications on his drawings, but the dimensions recorded by Saccardo,[6] 120–150 × 7–8 μ, fall well within the range observed by us and other workers.

Hartig's drawings, particularly where he illustrates typical conidia, an appendage, and the characteristic dark, thick-walled resting mycelium, and his description of the disease on cotyledons and other parts of maple seedlings, resulting in their death, indicate that his fungus is the same one considered by the following workers: Arnaud, Frank, Osterwalder, Westerdijk

[2] Hartig, Robert. Der Ahornkeimlingspilz, *Cercospora acerina* m. Untersuch. Forstbot. Inst. München **1**: 58–61. 1880.

[3] Frank, A. B. Krankheiten der Pflanzen. Band 2. 576 pp. Breslau. 1896.

[4] Arnaud, G. Le mildou des lilas et la maladie des cotyledons d'érable. Bull. Soc. Path. Veg. France **5**: 58–60. 1918.

[5] Osterwalder, A. Ueber die durch *Cercospora macrospora* Osterwalder verursachte Blattkrankheit bei den Pensees. Mitteil. der Thurg. Naturf. Gesells., Heft **25**: 59–80. 1924.

[6] Saccardo, P. A. Sylloge Fungorum **4**: 465. 1886.

and van Luijk,[7] Newhall, and ourselves. On the basis of priority, this fungus, with a list of known synonyms, therefore becomes:

Ansatospora acerina (Hart.) n. comb.

Cercospora acerina Hartig.
Sporidesmium acerina (Hart.) Frank.
Cercosporella acerina (Hart.) Arnaud.
Cercospora macrospora Osterwalder.
Cercospora cari Westerdijk and van Luijk.
Ansatospora macrospora (Ostw.) Newhall.

—H. N. HANSEN and C. M. TOMPKINS, Division of Plant Pathology, University of California, Berkeley, California.

The Culture Designated Madison 517 Identified as Polyporus tulipiferus.[1]—A fungus originally designated by the Forest Products Laboratory at Madison, Wisconsin, as *Fomes annosus* (Fries) Cooke, and later as Madison 517, has been used extensively in the United States for more than 20 years as one of the standard laboratory test organisms to determine relative toxicity of various wood preservatives, especially creosotes. Cartwright (see Robertson,[2] p. 33) was the first to suggest that this fungus was not *F. annosus.* From a comparison of cultures, and the evidence of a fruit body formed in culture, he stated that the fungus in question was *Polyporus tulipiferus* (Schw.) Overh. He did not describe the fruit body on which this conclusion was partially based. Cartwright and Findlay[3] later stated that it appeared to be identical with a culture of *P. tulipiferus* obtained from Canada, and Cartwright[4] in a subsequent publication states, in discussing this fungus, "in due course small fruit bodies were formed on beech blocks which appeared to be those of *P. tulipiferus.*" The fruit bodies were not described. Richards[5] compared Madison 517, *P. tulipiferus* 691, and typical cultures of *F. annosus,* and found that the two former had certain morphological and physiological characters in common, and differed in some respects from *F. annosus.* She considered the identity of Madison 517 to be uncertain.

The writers inoculated sections of cottonwood (*Populus deltoides* Marsh) and hackberry (*Celtis occidentalis* L.) with a culture of Madison 517 obtained from Dr. H. Schmitz, University Farm, St. Paul, who obtained his culture from Dr. Richards at the Forest Products Laboratory, and one of *Polyporus tulipiferus* isolated from a typical sporophore growing on a large

[7] Westerdijk, Johanna, and A. van Luijk. Eine Anthraknose des Kümmels (*Carum carvi*). Meded. Phytopath. Lab. Willie Commelin Scholten Baarn **8**: 51–54. 1924.

[1] Paper No. 2201, the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Robertson, W. A. Report of the Director of Forest Products Research for the year 1935. Rep. For. Prod. Res. Board, 1935. 1936.

[3] Cartwright, K. St. G. and W. P. K. Findlay. Principal decays of softwoods used in Great Britain. 106 pages. 1938.

[4] Cartwright, K. St. G. The relation between field and laboratory work in mycology. Trans. Brit. Myc. Soc. **22**: 222–238. 1939.

[5] Richards, C. Audrey. The doubtful identity of Fungus No. 517. Proc. Amer. Wood-Preservers Assoc. **33**: 104–106. 1937.

wound of a living hackberry in St. Paul. The method of inoculation was that described by Darley and Christensen.[6] Two to five months later the inoculated pieces of wood were placed under a greenhouse bench at 85° F. and watered occasionally. When fruit bodies began to appear, water was dripped slowly but continuously on the wood pieces, and within 3 to 4 weeks

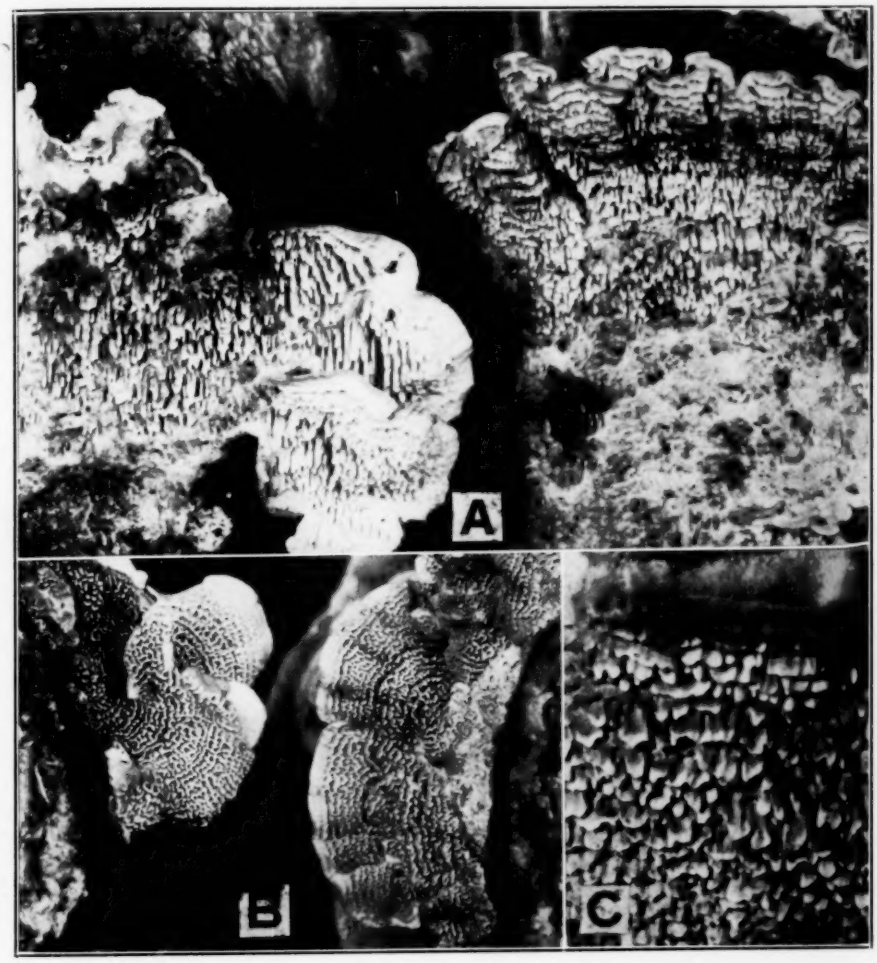

FIG. 1. A and B. Two views of the same fruit bodies. Note concentric arrangement in B. Left, *Polyporus tulipiferus;* right, Madison 517. C. Effuse-reflexed pileus of Madison 517.

thereafter the fruit bodies attained their full size. The wood was inoculated in August, 1943, and a considerable number of fruit bodies matured in August, 1944.

Macroscopically the fruit bodies of both isolates were typical of those formed by *Polyporus tulipiferus* in nature—resupinate to effused reflexed,

[6] Darley, Ellis F. and Clyde M. Christensen. An unusual sporophore of *Trametes suaveolens* produced on artificially inoculated wood. Phytopath. **33**: 328–330. 1943.

the upper surface white, villose, and faintly zoned, the under surface with irregular, daedaloid pores which soon broke up into concentrically arranged teeth (Fig. 1). Fruit bodies formed by Madison 517 were sent to Dr. L. O. Overholts, Pennsylvania State College, who identified them as *P. tulipiferus*.

Spore prints were obtained from fruit bodies of both isolates, and fresh pores and teeth were sectioned free hand, mounted in water, and the microscopic structures measured with the aid of a screw micrometer and an oil immersion lens. The average size of 60 cast spores of Madison 517 was 2.8×5.2 μ, with a range of $1.9–3.5 \times 4.1–6.5$ μ, while the average size of 60 cast spores of *Polyporus tulipiferus* was 2.8×4.9 μ with a range of $2.3–3.3 \times 3.7–6.4$ μ. Twenty-five basidia from each sporophore averaged 5.2 μ in diameter, the range in Madison 517 being 4.7–5.9 μ and in *P. tulipiferus* 4.7–5.2 μ. Cystidia were plentiful in the hymenia of fruit bodies of both isolates, and were typically encrusted, as is described for *P. tulipiferus;* the cystidia protruded up to 30 μ beyond the basidia in the fruit bodies of both isolates. The above evidence proves conclusively that what has hitherto been known as Madison 517 is *P. tulipiferus*.—ELLIS F. DARLEY, and CLYDE M. CHRISTENSEN, University Farm, St. Paul, Minnesota.